TECHNICAL APPENDICES FOR BARITE SOIL REMEDIATION GUIDELINES

Alberta
Environment

TECHNICAL APPENDICES FOR BARITE SOIL REMEDIATION GUIDELINES

Prepared by:
Axiom Environmental

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Alberta Environment
and
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Any comments, questions, or suggestions regarding the content of this document may be directed to:

Climate Change, Air, and Land Policy Branch
Alberta Environment
10th Floor, Oxbridge Place
9820 – 106th Street
Edmonton, Alberta T5K 2J6
Fax: (780) 422-4192

Additional copies of this document may be obtained by contacting:

Information Centre
Alberta Environment
Main Floor, Oxbridge Place
9820 – 106th Street
Edmonton, Alberta T5K 2J6
Phone: (780) 427-2700
Fax: (780) 422-4086
Email: env.infocent@gov.ab.ca

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Appendix I

BCLQAAC Report on Barium Analytical Methods

DEVELOPMENT OF BC CSR ANALYTICAL METHOD FOR SOLUBLE BARIUM

PHASE 1: EVALUATION OF VIABLE ANALYTICAL METHODS FOR SOLUBLE BARIUM

June 5, 2007

Prepared for:

BC Upstream Petroleum Environmental Task Group,
Barite-Barium Issues Subgroup

By:

BCLQAAC BARITE TASK GROUP

Mark Hugdahl,
Chair, BCLQAAC Technical Subcommittee,
ALS Laboratory Group, Environmental Division,
Vancouver, BC

Darlene Lintott,
Norwest Labs / Bodycote,
Edmonton, Alberta

Dr. John Ashworth,
ALS Laboratory Group, Environmental Division,
Edmonton, Alberta

Miles Tindal,
Axiom Environmental,
Calgary, Alberta

1.0 INTRODUCTION

The British Columbia Ministry of Environment is currently developing matrix numerical soil standards for barium. Barium soil standards will impact the operations of the BC oil and gas sector, because barite (barium sulfate) is regularly used as a weighting agent for drilling muds. The BC Upstream Petroleum Environmental Task Group – Barite Barium Issues Committee was formed to provide an inter-governmental and industry expert advisory body for collaboration on scientific and technical aspects needed to support the development of barium standards. Government representation on the Barite Barium Issues Committee includes BC MOE, the BC Oil and Gas Commission, and the BC Ministry of Energy, Mines, and Petroleum. Axiom Environmental and the Technical Sub-Committee of the British Columbia Laboratory Quality Assurance Advisory Committee (BCLQAAC) are represented on the committee in a scientific advisory capacity.

Because of the extremely low aqueous solubility and low toxicity of barite, the Barite-Barium Issues Committee is recommending that BC develop and endorse an analytical method for *soluble* barium for use by the Oil and Gas sector of BC at sites where detailed documentary evidence of barite usage exists. An appropriate analytical method for soluble barium should generate relatively low results for barite-barium in soils, but quantitative or near-quantitative results for more toxic forms of barium with higher aqueous solubilities and mobilities than barite, unless they are strongly sequestered onto soil cation exchange sites (e.g. barium chloride, barium acetate).

Prior to endorsing the regulation of soluble barium, BC MOE requires that development and validation of an appropriate method be conducted to ensure that it will be environmentally relevant and sufficiently protective for soluble forms of barium where they may be present.

The BCLQAAC Technical Subcommittee was tasked with the development and validation of a suitable analytical method for the determination of soluble barium in soils and solids. A task group was formed for this purpose, consisting of Mark Hugdahl (Chair, BCLQAAC Technical Sub-committee, ALS Laboratory Group), Darlene Lintott (Norwest Labs / Bodycote), and Dr. John Ashworth (ALS Laboratory Group). Miles Tindal (Axiom Environmental) also participated in an advisory capacity.

The BC CSR soluble barium method development project is being conducted in three stages:

- Phase 1: Evaluation of Viable Soluble Barium Analytical Methods
- Phase 2: Optimization and Ruggedness Testing of Analytical Method for Soluble Barium
- Phase 3: Interlaboratory Testing of Analytical Method for Soluble Barium

This report documents Phase 1 of the project.

2.0 PHASE 1 OUTLINE AND OBJECTIVES

Phase 1 of the project was divided into four components:

1. Identification of Potential Analytical Methods for Soluble Barium.
2. Preliminary Laboratory Testing of Candidate Methods for Soluble Barium.
3. Laboratory Optimization of Variables for Calcium Chloride Methods.
4. Cursory Evaluation of Methods for Total Barium.

For the first component, Miles Tindal prepared a summary of potential methods from the literature that were identified by the Barite-Barium Issues Committee and the BCLQAAC Barite Task Group. This summary is included in section 3 of this report.

The second component of the Phase 1 study consisted of a laboratory evaluation of all the proposed soluble barium methods using a barium carbonate field soil, a barite field soil, and three barite spiked background reference soils of differing soil textures.

The third component of the Phase 1 study involved laboratory optimizations of the calcium chloride extraction method that is prescribed by Alberta Environment for their regulation of soluble barium. This method was identified early on as a strong candidate for the BC method. Key variables associated with this method, including pH, calcium chloride concentration, and extraction ratio, were investigated in an attempt to characterize their effects on barite recovery from soils of varied textures.

The fourth component of Phase 1 was a preliminary evaluation of four different analytical methods for total and strong acid leachable barium. All samples analyzed by the targeted soluble barium methods were analyzed by these methods in order to determine the true total barium concentrations of the test samples, and also to assess the relative performance of these methods for total barium.

3.0 IDENTIFICATION OF POTENTIAL SOLUBLE BARIUM METHODS

There are a wide range of potential methods for the chemical analysis of barium in soil. Some of these methods may approximate the "total" barium concentration, and others may approximate the "soluble" barium concentration. A brief summary of five potential analytical methods for soluble barium is provided here, together with some discussion on the relevance and potential pros and cons of each.

Analytical methods for soils typically include an extraction step and a measurement step. In the case of determining soluble barium in soil, it is anticipated that the major difference between methods will be in the extraction, and that the measurement technique used (typically atomic absorption or inductively coupled plasma) will have relatively little effect on the outcome. Accordingly, this discussion focuses on extraction techniques. Extraction techniques reviewed here include the following:

- Alberta Barite Guidelines Calcium Chloride Extraction;
- DTPA extraction;
- EDTA extraction;
- Synthetic Precipitation Leaching Procedure (SPLP); and,
- Toxicity Characteristic Leaching Protocol (TCLP).

3.1 Alberta Barite Guidelines Calcium Chloride Extraction

This method uses excess calcium ions to displace barium from cation exchange sites, and measures the total amount of barium that is dissolved in the pore water together with the barium that can be released through cation exchange. The barium measured by this method has been shown to correlate well to the fraction of barium that is bioavailable to plants, while neither the fraction in a saturated paste extract, nor the strong acid leachable barium (by EPA 3051) in the soil yielded a satisfactory correlation with plant-bioavailable barium (Chevron Canada Ltd., unpublished data). The method was developed for the Alberta Barite Guidelines Project, and was shown to be rugged (not unduly sensitive to small variations in the extraction time, extraction methodology, or dilution ratio).

The extraction methodology may be summarized as follows. The sample is prepared by air drying and sieving to <2 mm. 10 g of prepared sample is added to 100 mL of 0.1 M calcium chloride solution, and is agitated for 2 hr on a platform shaker or end-over-end rotator. The extract is filtered to yield a clear filtrate, ready for analysis.

Among other variables, this project investigated whether a modification of this method using acidified calcium chloride solution was likely to be effective. The rationale was to simulate the most acid conditions that might reasonably be encountered in the environment in an oilfield setting (drilling mud sump, etc) without generating the extremely acid conditions of one of the strong acid extractions. A pH of 5 was identified by CAPP as approximately the worst case pH that would apply for these conditions.

Two other key variables of the $CaCl_2$ (calcium chloride concentration and extraction solution : soil ratio) were also evaluated to assess their effects on barium recovery from barite and a barium carbonate field soil.

3.2 DTPA Extraction (Carter, 1993)

DTPA is diethylene triamine pentaacetic acid, and is a chelating agent used in the extraction of available micronutrient cations Fe, Mn, Cu, and Zn. The DTPA soil test has potential relevance to the ecological direct contact exposure pathway, since it attempts to extract the labile fraction of various trace element cations in soil, and the results have been correlated to the fraction that is bioavailable to plants. The DTPA method was first proposed by Lindsay and Norvell (1967), and has received extensive validation (ten published papers referenced in Lindsay and Norvell, 1978) as being a good indicator of plant-available Zn, Fe, Mn, and Cu. The DTPA methodology published by Lindsay and Norvell (1978) and repeated in Carter (1993) was optimized for the extraction of the trace elements Zn, Fe, Mn, and Cu in soil. DTPA is used in this extraction because it has a favourable combination of stability constants for forming complexes with the four above-noted ions (Lindsay and Norvell, 1978). The DTPA method is inexpensive, reproducible, and easily adaptable to routine operations (Carter, 1993). No record was found of the method having been used to estimate plant-available barium.

The DTPA soil test was based on sound principles of soil and chelation chemistry (Lindsay and Norvell, 1978), and the extraction fluid is buffered to pH 7.3. The applicability of the method at more acidic pH values may be limited due to the potential for the DTPA to become saturated by calcium freed from the dissolution of calcium carbonate and bicarbonate. Detailed chemical speciation modelling would likely be required before attempting to adapt the parameters of the extracting solution.

The standard (Carter, 1993) DTPA soil test uses the following procedure. 10 g of air-dry soil (2 mm) is placed in a 125 mL Erlenmeyer flask. 20 mL of the extracting solution (0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M triethanolamine) is added, and the covered flask is agitated for 2 hours on a horizontal shaker with a stroke of 8.0 cm, and a speed of 120 cycles/min. The resultant suspension is filtered through Whatman No. 42 filter paper prior to analysis. The purpose of the triethanolamine (TEA) is to buffer the extracting solution to pH 7.3. The purpose of the $CaCl_2$ is to enable the extract to attain equilibrium with $CaCl_2$, and so minimize the dissolution of $CaCO_3$.

Before using this method as a possible candidate to assess available barium, it would be necessary to ensure that there was sufficient chelation capacity for the maximum concentrations of total labile cations that were anticipated. It should be noted that with the standard DTPA method, the maximum theoretical concentration of total extracted cations would be 10 mM cations/kg soil, equivalent to 1,370mg/kg soluble barium, if barium was the only cation extracted. The concentration of soluble barium in soil may exceed this value in certain situations, and accordingly, it may be necessary to increase either the concentration of the DTPA in the extracting solution, or the volume of extracting solution. Using 5 g soil and 25 mL of extracting solution with 0.05M DTPA would allow a maximum theoretical concentration of 250 mM of cations to be extracted, equivalent to approximately 34,000 mg/kg soluble barium, if barium was the only cation extracted.

It would also be possible to buffer the extracting solution to a more acidic pH, but, in a calcareous soil, this would likely result in increased dissolution of $CaCO_3$, with the potential for saturating the available DTPA molecules with Ca, rather than Ba. As noted above, chemical speciation modelling would be required to ensure that the theoretical basis of the DTPA soil test was still valid for the amended composition of the extracting solution.

3.3 EDTA Extraction (Carter, 1993)

EDTA is ethylene diamine tetraacetic acid, and is a chelating agent similar in structure to DTPA. Many of the comments made for the DTPA soil test above apply equally to the EDTA extraction. EDTA extractable Cd, Cu, Pb, and Zn has been shown to be a good measure of the labile fraction of these soils that is available to plants (Merry and Tiller, 1978). Williams and Thornton (1973) have also shown that EDTA-extractable Mo and Se give a good prediction of the plant-available fraction of these metals, while total Mo and Se correlated poorly with the plant-available fractions. As with the DTPA soil test, the EDTA method has potential relevance to the ecological direct contact exposure pathway.

Various extraction procedures using EDTA have been described. These vary in the concentration of EDTA used, and the buffered pH of the extracting solution. Carter (1993) describes the following procedure. 5 g of air-dry soil (2 mm) is placed in a 125 mL Erlenmeyer flask. 25 mL of the extracting solution (0.05 M EDTA, adjusted to pH 7.0 with NH_4OH) is added, and the covered flask is shaken for 1 hour at a speed of 120 cycles/min. The resultant suspension is filtered through Whatman No. 42 filter paper prior to analysis.

Using the Carter (1993) EDTA method, the maximum theoretical concentration of total extracted cations would be 250 mM cations/kg soil, equivalent to approximately 34,300 mg/kg soluble barium, if barium was the only cation extracted.

3.4 Synthetic Precipitation Leaching Procedure (SPLP) USEPA SW-846 Method 1312

This method is included as a possible option for measuring “soluble” barium. The extraction fluid is designed to be representative of acid rain in the western United States. This method would be expected to be significantly less aggressive than strong acid extractions.

The extraction methodology may be summarized as follows. The particle size of the soil phase is reduced, if necessary. A minimum sample of 100 g is extracted (18 hours on a rotary agitator) with an amount of extraction fluid equal to 20 times the weight of the solid phase. For sites west of the Mississippi, the extraction fluid used is a 60/40 weight percent mixture of sulfuric and nitric acids with a pH of 5.0. Following extraction, the sample is then filtered ready for analysis.

The SPLP method has potential relevance to calculating soil quality guidelines protective of groundwater exposure pathways, since it aims to simulate the leaching of soils by through the action of a mildly acidic synthetic precipitation.

3.5 Toxicity Characteristic Leaching Procedure (TCLP) USEPA SW-846 Method 1311

This method is included for completeness, since it is commonly used in the environmental field to determine “leachable” metals in soils and industrial wastes. The procedure is a requirement within many landfill disposal regulations, including the BC Hazardous Waste Regulation.

The methodology is similar to the SPLP, but the extraction fluid used is different (slightly more acidic). In the case of drilling waste sumps, this method may be less relevant than the SPLP, based on the extraction fluid.

The extraction methodology may be summarized as follows. The particle size of the soil phase is reduced, if necessary. A minimum sample of 100 g is extracted (18 hours on a rotary agitator) with an amount of extraction fluid equal to 20 times the weight of the solid phase. Depending on the characteristics of the sample, the extraction fluid used is either acetic acid with a pH of 2.88, or a mix of acetic acid and sodium hydroxide with a pH of 4.93 (most sample types require the pH 4.93 fluid). Following extraction, the sample is filtered prior to analysis.

The TCLP extraction has been suggested by some as possibly being relevant to determining the available fraction of a metal in a mammalian stomach, based on the pH of the extraction. However, while the pH may be close to the physiologically relevant range, the extraction ratio is not, since incidental soil ingestion in the human stomach likely results in an extraction ratio closer to 10,000:1. True physiologically-based extraction tests (PBETs) have shown that the fraction of barium extracted from a barite-contaminated soil is strongly dependent on the extraction ratio.

4.0 EXPERIMENTAL PLAN FOR LABORATORY STUDIES

The primary objective of the phase 1 laboratory studies was to assess the various candidate soluble barium methods for their efficacy and recovery when applied to barite impacted soils of various textures. It is important to note that the intent of a soluble barium method is to be able to discriminate between soluble and insoluble forms of barium. Thus an ideal method for soluble barium will achieve quantitative or near quantitative recoveries of soluble and non-sequestered forms of barium, with far lesser recovery of barite barium.

Two field-collected barium-affected soils (a barium carbonate soil, and a barite soil) and three barite-spiked background reference soils were prepared for analysis by a series of analytical methods for soluble barium and total barium ranging widely in vigour. The background reference soils included a sand, a sandy clay loam, and a clay. These were freshly spiked with drilling-mud grade barite at a concentration of 2,000 mg/kg barium (above background levels).

4.1 PREPARATION OF TEST SOILS

Five test soils were included in the Phase 1 investigations. Two of the soils were field-collected soils provided by ALS Edmonton. Both soils were assumed to be contaminated with barium from their original drilling waste related field sites. The remaining three soils, provided by Husky, were collected from sites as background samples and have likely not been exposed to industrial contaminants. These field background soils, which had previously been dried and ground, were spiked with barite to achieve desired barium concentrations of 2,000 mg/kg.

The first field soil used in the study was a sandy loam, with a total barium concentration of 2,460 mg/kg (by Fusion-XRF and Direct-XRF). The site in question had used barium carbonate as a weighting agent (not a common practice). Hence, this soil was designated as "Barium Carbonate Field Soil". This soil provided a valuable opportunity to assess candidate soluble barium methods using a soil that had been impacted with a more soluble form of barium than barite. It is important to note that this soil may contain a mixture of barium carbonate and barite.

A second field soil was obtained that is representative of typical barite sites. The second field soil was also a sandy loam with approximately 2,600 mg/kg total barium (by both Fusion-XRF and Fusion-ICP methods). Soluble barium in this sample by the Alberta Environment $CaCl_2$ method was typical of barite contaminated soils associated with drilling wastes. This soil was designated as "Barite Field Soil".

In order to investigate the impact of soil texture on the soluble barium methods, three barite spiked background soils were included in this study. The background soils types included sand, clay, and a sandy clay loam. These soils were characterized extensively for physical and chemical parameters (presented in Appendix 1). Key characteristics of the three soils that are most pertinent to soluble barium methods are provided in Table 1.

Table 1. Primary Characteristics of Background Soils

Parameter Name	Units	"BaCO3" Soil (Sandy Loam)	"Barite" Soil (Sandy Loam)	Sand	Clay	Silty Clay Loam
Sand	%	69.4	55.6	96.4	14.4	45.7
Silt	%	14.8	24.8	1.2	26.2	25.3
Clay	%	15.8	19.6	2.4	59.4	29
CEC	meq/100g			1.3	24.6	12.6
Barium (EPA 3050B)	mg/kg			64	333	195
Extractable Barium	mg/kg			9.4	1.1	2.7
Sulfate-S	mg/kg			3.4	1,120	326

The soils were prepared by drying at 30°C, then disaggregating to <2 mm (but were not pulverized or sieved). Soils were spiked with a supply of barite (Canamara United Supply, provided by Newalta) commonly used in drilling muds. Barite powder was added directly to the dry background soils to achieve a target concentration of 2,000 mg/kg (as barium) above background barium levels. The barium background levels in these soils are given in table 1. The first batch of barium spiked soils, used for replicate analysis 1 and 2 for all studies was mixed using end-over-end rotation for one hour. The batch was then split into two replicate samples and distributed for testing. A second batch of barite spiked soils was also prepared but was mixed by rotation for 24 h to ensure homogeneity. This batch was used as a third replicate for the extraction studies.

4.2 STRONG ACID AND TOTAL BARIUM TEST METHODS USED

Four methods for measuring total or strong acid leachable barium were included in this study. The USEPA 3050B Method is a strong acid digestion method, followed by ICP analysis. It is one of the most commonly employed digestion methods in North America for the analysis of environmentally available metals in soils. The BC SALM method is another strong acid digestion technique, and is used specifically for analysis of "total" metals in soils under the BC Contaminated Sites Regulations as directed by the Ministry of the Environment. Fusion, or "whole rock analysis" is a dissolution technique commonly employed for geochemical analysis. It is not normally applied for environmental analysis, because constituents of rock matrices are not considered environmentally available. Fusion-X-Ray Fluorescence (Fusion-XRF) is an analysis method that combines the Fusion dissolution technique with XRF quantitation, resulting in a total metals assay with an extremely high analytical concentration range for barium and other major compositional elements.

These tests were included to give us true measures of the total barium in our test samples, and to examine the limitations of each method for measuring high concentrations of barite barium in soil. Previous work conducted on drilling waste contaminated soils by Norwest Labs has indicated that the strong acid digest methods have a maximum or upper limit of barium that can be extracted from soil (estimated between 5,000 to 8,000 mg/Kg Ba), but correlation of these maxima with true total barium concentrations are not known. Both Fusion-ICP and Fusion-XRF are considered likely to achieve true estimates of total barium in soils, including highly bound or complexed barium species.

4.2.1 US EPA 3050B

Modified versions of the US EPA 3050B method were used by both Norwest / Bodycote Edmonton (NWL) and ALS Edmonton (ALS-EDM) for total barium analysis. Samples are normally dried, disaggregated (e.g. by flail grinder), and sieved prior to digestion, with the >2mm fraction discarded (spiked samples in this study were not sieved).

Soil sub-samples are digested with concentrated nitric acid, with the addition of hydrogen peroxide. Neither NWL or ALS-EDM used hydrochloric acid in this digestion, which is an option within the 3050B method that might also be expected to influence recoveries. Digestion times and temperatures varied slightly between the two labs.

Both NWL and ALS digested 0.5 g soil weights, with dilution to a final volume of 50 mL. ALS used a digestion temperature of 95C. NWL used a digestion temperature of 120C. Exact weight and digestion volume requirements are not prescribed by the 3050B method. For barite analyses, these variables may results in significant differences among laboratories.

4.2.2 BC Strong Acid Leachable Metals (SALM)

Variations of the BC SALM strong acid digestion method were used by NWL, ALS Vancouver (ALS-VAN), and ALS-EDM for selected samples in the study. Samples are normally dried, ground, and sieved prior to digestion, with the >2mm fraction discarded (spiked samples in this study were not sieved).

The BC SALM method is a performance based method. Reference conditions are specified, and known critical elements are prescribed, but labs may otherwise make changes to the method where equivalence is demonstrated. However, the effects of changes to digestion variables on recoveries of barite may be more pronounced than on typical soil samples.

The BC SALM reference method specifies digestion for 2 hours at 90+/-5°C using a 1:1 ratio of nitric and hydrochloric acids, with at least 5mL of concentrated 1:1 HCl:HNO3 acid mixture per gram of sample. Digestion time and temperature are not prescribed elements of the method, and may be varied if equivalence is demonstrated. Digests are typically diluted to a final volume of 50 mL prior to analysis by ICP for barium and other metals.

ALS-VAN digested approximately 1.5 gram sub-samples with 10 mL of concentrated 1:1 nitric:hydrochloric acids. Samples were digested for 2 hours at 90°C, as per the BC SALM reference digestion conditions. Digests were diluted to 50 mL prior to ICP analysis.

NWL digested 0.5 gram sub-samples with 5 mL of concentrated 1:1 nitric:hydrochloric acids. Samples were digested for 4 hours at 120°C (a performance based modification of the method). Digests were diluted to 50 mL prior to ICP analysis.

4.2.3 Fusion (Lithium Borate) - ICP

The Fusion - ICP method is applicable to whole rock analysis of soils, coal, ash and cement products, and was conducted by Norwest / Bodycote Laboratories in Surrey. Soils were prepared by drying and grinding to < 75 um, then ashing at 900 C. The ash is mixed with a borate flux (Lithium metaborate and lithium tetraborate) in a graphite crucible and fused at 1050°C for 40 minutes. The melt is dissolved in HCl and HNO_3 for 1 hour with agitation. Barium is measured in the digest by ICP.

4.2.4 Fusion - X-Ray Fluorescence (Fusion-XRF)

ALS Chemex (North Vancouver, BC) conducted analysis of the test soils for total barium by Fusion X-Ray Fluorescence (XRF). In this method, the solid sample is first digested using the metaborate Fusion dissolution technique. Barium analysis is then conducted by XRF.

In XRF, a homogeneous sub-sample of either a pulverized and pelletized soil, or in this study, of a Fusion digest, is irradiated with x-rays. When an atom absorbs these x-rays, low energy electrons may be dislodged from the innermost shells of the atom, creating vacancies which are then filled by electrons from higher energy outer electron shells. This transition radiates energy in the form of x-rays with wavelengths that are characteristic of the given atom. Measurement of the intensity of the x-ray fluorescence emitted at specific wavelengths can be used to quantify the concentration of the atom in the sub-sample.

EPA SW-846 Method 6200 is one official reference method for the XRF technique. Method 6200 describes a portable screening procedure for field quantitation of selected metals in soils, including barium. However, field application of XRF may be prone to inaccuracies and interferences, and has a limited upper concentration range. The barium carbonate field sample was analyzed for total barium using a portable XRF instrument, and excellent confirmation was achieved versus the Fusion-XRF technique.

Laboratory applications of the method in combination with the Fusion dissolution technique do not suffer from inhomogeneity problems, and can have a range of up to at least 50% barium.

4.3 CALCIUM CHLORIDE EXTRACTABLE BARIUM METHODS USED

4.3.1 Alberta Environment Extractable Barium Method

The standard Alberta Environment method employs 0.1 M $CaCl_2$ as the extraction fluid. The dried and disaggregated samples were extracted using 5 g of sample with 50 mL extraction fluid, with agitation for 2 hours. The extract was filtered through a paper filter and analyzed for barium by ICP. Extractable barium results by this standard Alberta method are referred to as AENV 10:1 0.1 M $CaCl_2$ in Table 3.

4.3.2 Acidic and Basic Calcium Chloride Extractions

A study was conducted to investigate whether modification of pH of the calcium chloride solution has an impact on measured soluble barium concentrations. Extractions for soluble barium were completed using the same standard AENV barium method as described above, but the 0.1M $CaCl_2$ extraction fluid was adjusted to pH 3 and pH 10 using dilute HCl or NaOH. The extraction solutions were allowed to settle overnight and the decanted supernatant was analyzed for barium. No attempts were made to buffer the pH of these extraction solutions, which was considered beyond the scope of this study.

4.3.3 Calcium Chloride Extractable Barium with Increased Extraction Ratio

Previous work with drilling waste contaminated soil has demonstrated that variation of sample size and extraction solution volume can have a major impact on the concentration of both total and soluble barium measurements. This effect is likely a result of the extremely low aqueous solubility of barium sulfate. The relative concentrations of anions present in solution, and sulfate in particular, tend to control the concentration of barium in solution. The impact of extraction volume was examined using the AENV soluble barium method, but doubling the volume of 0.1 M $CaCl_2$, to achieve a 20:1 extraction fluid to soil ratio. The results of these tests are referred to in Table 3 as 20:1 $CaCl_2$.

4.3.4 Extractable Barium with Modified Calcium Chloride Concentration

The principle behind the AENV soluble barium method is that an increased concentration of barium in the extraction solution occurs because barium cations that are sorbed to the soil can be solubilized when excess calcium ions exchange with barium (and other cations) on the cation exchange sites of clay particles. The cation exchange mechanism should have minimal effect on insoluble barite salts, which should not interact with cation exchange sites because they are uncharged (unless they become solubilized).

The Methods Task Group speculated that increasing the concentration of calcium may increase the recoveries of soluble barium from soils with higher cation exchange potential. This possibility was investigated in a series of tests using the AENV Barite Method, but using variable calcium chloride concentrations for the extraction fluids. Extractions for the test soils (variable clay content and cation exchange capacity) were completed using 0.05 and 1.0M $CaCl_2$, in addition to the standard 0.1M concentration.

4.3.5 Extractable Barium with Increased $CaCl_2$ Concentration & Extraction Ratio

The combined effect of increased extraction volume and increased calcium chloride concentration was also examined by performing 20:1 $CaCl_2$:soil extracts with 1.0M $CaCl_2$.

4.4 ALTERNATIVE EXTRACTION METHODS USED

In addition to the calcium chloride methods, several alternative extraction methods were examined for their ability to capture soluble barium from soils. These methods were selected as they are commonly used for analysis of metals for other specific assessment or regulatory purposes.

4.4.1 EPA 1311 – Toxicity Characteristic Leaching Procedure (TCLP)

The TCLP method (USEPA SW-846 Method 1311) was performed by weighing 10 g of test sample into 500 mL polypropylene bottles and adding 200 mL of extraction fluid to achieve a 20:1 extraction ratio. 10 gram sub-samples were used because the test samples were highly homogeneous and were limited in quantity. The extraction fluid was prepared by addition of acetic acid and sodium hydroxide to achieve a pH of 4.93. Samples were extracted for 18 h using a rotary agitator. The fluid was filtered with a Whatman #42 filter paper, and the final pH was recorded. Analysis for barium was conducted by ICP-MS.

4.4.2 EPA 1312 – Synthetic Precipitation Leaching Procedure (SPLP)

The SPLP extraction fluid was prepared by addition of a 60/40 mixture of sulfuric and nitric acids to deionized water to a pH of 4.2. The test soils were extracted at a leachate ratio of 20:1, using 10 g of soil and 200 mL of extraction fluid. 10 gram sub-samples were used because the test samples were highly homogeneous and were limited in quantity. The extraction was done for 18 h on a rotary agitator. The extraction fluid was then filtered with a Whatman paper filter, and the pH of the final solution was measured. Analysis for barium was conducted by ICP-MS.

4.4.3 Chelation Methods - DTPA and EDTA

The DTPA and EDTA solutions used for this study employed the standard chelation agent concentrations, as outlined in the Carter methods. The DTPA solution was 0.005 M DTPA in 0.01 M CaCl2 and 0.1 M TEA. The EDTA solution was 0.05 M Na_2-EDTA, adjusted to pH 7 with an ammonia solution. Test soils by both methods were extracted using 5 g of soil with 25 mL of extraction solution in polyethylene vials, with end-to-end shaking for 1 hour. The vials were allowed to settle overnight, then the supernatants were analyzed for barium by ICP.

As indicated in section 3.3, the chelate concentrations used will limit the amount of barium that could potentially be complexed. In the absence of other competing cations, the theoretical maximum chelated barium concentrations by the methods as employed in this study would be 3,430 mg/kg for DTPA and 34,300 mg/kg for EDTA. Of course barium is not the only cation in competition for chelating agents, and other cations may form more stable complexes with EDTA, so the actual maxima will be lower.

5.0 RESULTS AND DISCUSSION

Analytical results for strong acid and total barium methods are provided below in Table 2. Results for calcium chloride extraction methods are presented in Table 3. Results for the alternative extraction methods are shown in Table 4.

Table 2. Strong Acid and Total Barium Methods

Test Sample	Data Type	EPA 3050 Ba	EPA 3050 Ba	BC SALM	BC SALM	BC SALM	Fusion-XRF Total Ba	Fusion-ICP Total Ba
		NWL Edm.	ALS Edm.	NWL Edm.	ALS Edm.	ALS Vanc.	ALS Chemex	NWL Surrey
$BaCO_3$ Field Soil	Rep. 1	1,560	1,420	1,550	1,500	1,710	2,400	
	Rep. 2		1,510			1,720	2,075*	
	Rep. 3					1,680	2,900*	
	Mean	**1,560**	**1,465**	**1,550**	**1,500**	**1,703**		
Clay + Barite (2,000 mg/kg Ba spike)	Rep. 1	1,170	728	1,160		366	3,000	2,750
	Rep. 2	1,210	636	1,130		295	2,400	
	Rep. 3	1,760		540		285		
	Mean	**1,380**	**682**	**943**		**315**		
Sandy Clay Loam + 2,000 mg/kg Barite	Rep. 1	1,680	1,050	1,640		608	2,600	2,740
	Rep. 2	1,970	1,090	1,770		412	2,700	
	Rep. 3	2,000		1,430		597		
	Mean	**1,883**	**1,070**	**1,613**		**539**		

4.4.2 EPA 1312 – Synthetic Precipitation Leaching Procedure (SPLP)

The SPLP extraction fluid was prepared by addition of a 60/40 mixture of sulfuric and nitric acids to deionized water to a pH of 4.2. The test soils were extracted at a leachate ratio of 20:1, using 10 g of soil and 200 mL of extraction fluid. 10 gram sub-samples were used because the test samples were highly homogeneous and were limited in quantity. The extraction was done for 18 h on a rotary agitator. The extraction fluid was then filtered with a Whatman paper filter, and the pH of the final solution was measured. Analysis for barium was conducted by ICP-MS.

4.4.3 Chelation Methods - DTPA and EDTA

The DTPA and EDTA solutions used for this study employed the standard chelation agent concentrations, as outlined in the Carter methods. The DTPA solution was 0.005 M DTPA in 0.01 M CaCl2 and 0.1 M TEA. The EDTA solution was 0.05 M Na_2-EDTA, adjusted to pH 7 with an ammonia solution. Test soils by both methods were extracted using 5 g of soil with 25 mL of extraction solution in polyethylene vials, with end-to-end shaking for 1 hour. The vials were allowed to settle overnight, then the supernatants were analyzed for barium by ICP.

As indicated in section 3.3, the chelate concentrations used will limit the amount of barium that could potentially be complexed. In the absence of other competing cations, the theoretical maximum chelated barium concentrations by the methods as employed in this study would be 3,430 mg/kg for DTPA and 34,300 mg/kg for EDTA. Of course barium is not the only cation in competition for chelating agents, and other cations may form more stable complexes with EDTA, so the actual maxima will be lower.

5.0 RESULTS AND DISCUSSION

Analytical results for strong acid and total barium methods are provided below in Table 2. Results for calcium chloride extraction methods are presented in Table 3. Results for the alternative extraction methods are shown in Table 4.

Table 2. Strong Acid and Total Barium Methods

Test Sample	Data Type	EPA 3050 Ba	EPA 3050 Ba	BC SALM	BC SALM	BC SALM	Fusion-XRF Total Ba	Fusion-ICP Total Ba
		NWL Edm.	ALS Edm.	NWL Edm.	ALS Edm.	ALS Vanc.	ALS Chemex	NWL Surrey
$BaCO_3$ Field Soil	Rep. 1	1,560	1,420	1,550	1,500	1,710	2,400	
	Rep. 2		1,510			1,720	2,075*	
	Rep. 3					1,680	2,900*	
	Mean	**1,560**	**1,465**	**1,550**	**1,500**	**1,703**	[redacted]	
Clay + Barite (2,000 mg/kg Ba spike)	Rep. 1	1,170	728	1,160		366	3,000	2,750
	Rep. 2	1,210	636	1,130		295	2,400	
	Rep. 3	1,760		540		285		
	Mean	**1,380**	**682**	**943**		**315**	[redacted]	[redacted]
Sandy Clay Loam + 2,000 mg/kg Barite	Rep. 1	1,680	1,050	1,640		608	2,600	2,740
	Rep. 2	1,970	1,090	1,770		412	2,700	
	Rep. 3	2,000		1,430		597		
	Mean	**1,883**	**1,070**	**1,613**		**539**	[redacted]	[redacted]

Test Sample	Data Type	EPA 3050 Ba	EPA 3050 Ba	BC SALM	BC SALM	BC SALM	Fusion-XRF Total Ba	Fusion-ICP Total Ba
		NWL Edm.	ALS Edm.	NWL Edm.	ALS Edm.	ALS Vanc.	ALS Chemex	NWL Surrey
Sand + Barite (2,000 mg/kg Ba spike)	Rep. 1	1,840	1,360	1,030		630	2,800	2,580
	Rep. 2	2,230	1,410	2,280		623	(1,500)**	
	Rep. 3	2,040		2,040		685		
	Mean	**2,037**	**1,385**	**1,783**		**646**	[illegible]	[illegible]
Barite Field Soil	Rep. 1	2,550	2,100	2,000		1870	2,600	2,530
	Rep. 2	2,190	1,810	2,390		1830	2,600	
	Rep. 3					1830		
	Mean	**2,370**	**1,955**	**2,195**		**1843**	[illegible]	[illegible]

All units in mg/kg. Red highlighted results are true total barium estimates.

* = Direct measurement XRF by a portable instrument (no fusion step) confirmed the 2,400 mg/kg fusion-XRF value.

**( ) = Outlier, excluded from averages.

Third replicate is an independently prepared barite spiked sample, equilibrated prior to digestion by rotation for 24h.

Table 3. Calcium Chloride Extraction Methods

Test Sample	Data Type	0.05 M $CaCl_2$ 10:1 Extr. pH=3	0.05 M $CaCl_2$ 10:1 Extr. pH=7	0.05 M $CaCl_2$ 10:1 Extr. pH=10	0.1M $CaCl_2$ 10:1 Extr. pH=7 AENV	0.1M $CaCl_2$ 10:1 Extr. pH=7 AENV	0.1M $CaCl_2$ 10:1 Extr. pH=3	0.1M $CaCl_2$ 20:1 Extr. pH=7 18hr/2hr	1.0M $CaCl_2$ 10:1 Extr. pH=7 2 hr	1.0M $CaCl_2$ 20:1 Extr. pH=7 2hr
		ALS	**ALS**	**ALS**	**ALS**	**NWL**	**ALS**	**NWL**	**ALS**	**NWL**
$BaCO_3$ Soil	Rep. 1				339	338	353	424	440	462
	Rep. 2				351		352	407		528
	Rep. 3				344		363	411		
	Mean				345	338	356	414	440	495
Clay + 2,000 mg/kg Barite	Rep. 1	0.7	0.7	0.7	1.2	2.0		5.2	14.6	41.0
	Rep. 2	0.7	0.7	0.7	1.1	1.4		3.6		30.8
	Rep. 3					2.4		4.6		35.3
	Mean	0.7	0.7	0.7	1.2	1.9		4.5	14.6	35.7
Sandy Clay Loam + 2,000 mg/kg Barite	Rep. 1	1.8	1.8	2.0	3.3	3.7		9.7	28.2	54.4
	Rep. 2	1.8	1.9	1.9	3.3	4.2		10		53.1
	Rep. 3					5.1		10		57.0
	Mean	1.8	1.9	1.9	3.3	4.3		9.9	28.2	54.8
Sand + 2,000 mg/kg Barite	Rep. 1	47.5	50.4	45.4	66.8	65.7		136	163	285
	Rep. 2	53.7	48.9	44.2	66.2	65.7		136		301
	Mean	50.6	49.7	44.8	66.5	65.7		136	163	293
Barite Field Soil	Rep. 1	21.0	19.9	19.9	37.8	34.5		66.9	106	189
	Rep. 2	20.9	20.2	19.8		31.7		67.0		189
	Rep. 3									
	Mean	21.0	20.1	19.9	37.8	33.1		67.0	106	189

All units in mg/kg.

ALS = ALS Edmonton

NWL = Norwest Labs / Bodycote Edmonton

Test Sample	Data Type	EPA 3050 Ba	EPA 3050 Ba	BC SALM	BC SALM	BC SALM	Fusion-XRF Total Ba	Fusion-ICP Total Ba
		NWL Edm.	ALS Edm.	NWL Edm.	ALS Edm.	ALS Vanc.	ALS Chemex	NWL Surrey
Sand + Barite (2,000 mg/kg Ba spike)	Rep. 1	1,840	1,360	1,030		630	2,800	2,580
	Rep. 2	2,230	1,410	2,280		623	(1,500)**	
	Rep. 3	2,040		2,040		685		
	Mean	**2,037**	**1,385**	**1,783**		**646**		
Barite Field Soil	Rep. 1	2,550	2,100	2,000		1870	2,600	2,530
	Rep. 2	2,190	1,810	2,390		1830	2,600	
	Rep. 3					1830		
	Mean	**2,370**	**1,955**	**2,195**		**1843**		

All units in mg/kg. Red highlighted results are true total barium estimates.

* = Direct measurement XRF by a portable instrument (no fusion step) confirmed the 2,400 mg/kg fusion-XRF value.

**( ) = Outlier, excluded from averages.

Third replicate is an independently prepared barite spiked sample, equilibrated prior to digestion by rotation for 24h.

Table 3. Calcium Chloride Extraction Methods

Test Sample	Data Type	0.05 M $CaCl_2$ 10:1 Extr. pH=3	0.05 M $CaCl_2$ 10:1 Extr. pH=7	0.05 M $CaCl_2$ 10:1 Extr. pH=10	0.1M $CaCl_2$ 10:1 Extr. pH=7 AENV	0.1M $CaCl_2$ 10:1 Extr. pH=7 AENV	0.1M $CaCl_2$ 10:1 Extr. pH=3	0.1M $CaCl_2$ 20:1 Extr. pH=7 18hr/2hr	1.0M $CaCl_2$ 10:1 Extr. pH=7 2 hr	1.0M $CaCl_2$ 20:1 Extr. pH=7 2hr
		ALS	ALS	ALS	ALS	NWL	ALS	NWL	ALS	NWL
$BaCO_3$ Soil	Rep. 1				339	338	353	424	440	462
	Rep. 2				351		352	407		528
	Rep. 3				344		363	411		
	Mean				345	338	356	414	440	495
Clay + 2,000 mg/kg Barite	Rep. 1	0.7	0.7	0.7	1.2	2.0		5.2	14.6	41.0
	Rep. 2	0.7	0.7	0.7	1.1	1.4		3.6		30.8
	Rep. 3					2.4		4.6		35.3
	Mean	0.7	0.7	0.7	1.2	1.9		4.5	14.6	35.7
Sandy Clay Loam + 2,000 mg/kg Barite	Rep. 1	1.8	1.8	2.0	3.3	3.7		9.7	28.2	54.4
	Rep. 2	1.8	1.9	1.9	3.3	4.2		10		53.1
	Rep. 3					5.1		10		57.0
	Mean	1.8	1.9	1.9	3.3	4.3		9.9	28.2	54.8
Sand + 2,000 mg/kg Barite	Rep. 1	47.5	50.4	45.4	66.8	65.7		136	163	285
	Rep. 2	53.7	48.9	44.2	66.2	65.7		136		301
	Mean	50.6	49.7	44.8	66.5	65.7		136	163	293
Barite Field Soil	Rep. 1	21.0	19.9	19.9	37.8	34.5		66.9	106	189
	Rep. 2	20.9	20.2	19.8		31.7		67.0		189
	Rep. 3									
	Mean	21.0	20.1	19.9	37.8	33.1		67.0	106	189

All units in mg/kg.

ALS = ALS Edmonton

NWL = Norwest Labs / Bodycote Edmonton

Table 4. Alternative Extraction Methods

Test Sample	Data Type	Deionized Water 20:1 Extraction	TCLP 20:1 Leachate pH 4.93	SPLP 20:1 Leachate	EDTA Extraction 0.05M	DTPA Extraction 0.005M
		NWL Edm.	NWL Edm.	NWL Edm.	ALS Edm.	ALS Edm.
$BaCO_3$ Soil	Rep. 1	2.0	108	6.2	484	19.2
	Rep. 2		104	6.0	482	18.8
	Rep. 3		98	5.0	496	19.3
	Mean	2.0	103	5.8	487	19.1
Clay + Barite (2,000 mg/kg Ba spike)	Rep. 1		1.8	0.6	0.99	0.04
	Rep. 2		0.8	0.4	0.98	0.03
	Mean		1.3	0.5	0.99	0.04
Sandy Clay Loam + Barite (2,000 mg/kg Ba spike)	Rep. 1		1.4	0.6	2.5	0.18
	Rep. 2		1.5	0.6	3.0	0.14
	Mean		1.5	0.6	2.8	0.16
Sand + Barite (2,000 mg/kg Ba spike)	Rep. 1		62.2	1.0	56.1	3.6
	Rep. 2		57.8	1.0	56.7	3.5
	Mean		60.0	1.0	56.4	3.6
Barite Field Soil	Rep. 1		42.8	0.6	9.83	0.80
	Rep. 2		44.0	0.6	9.81	0.77
	Mean		43.4	0.6	9.82	0.79

All units in mg/kg.

5.1 Results for Total Barium Methods

Both the Fusion-XRF and Fusion-ICP methods appeared to recover essentially 100% of the expected target concentrations of barium in all test samples. Exact recoveries for total barium in the field spiked samples could not be determined, because no Fusion-XRF or Fusion-ICP data was available for the unspiked soils (only strong acid barium data was available for background concentrations). Using the strong acid barium data as the background soil barium concentrations, average recoveries by the Fusion-XRF and Fusion-ICP methods were 117%, 122%, and 124% for the spiked clay, sandy clay loam, and sand samples respectively. This implies that the true total barium concentrations in these samples are likely about 400–500 mg/kg higher than the strong acid results (see Appendix 1) indicate.

The Fusion-XRF and Fusion-ICP methods were in very close agreement, except for one outlying Fusion-XRF replicate on the sand sample. It is suspected that this replicate may have been low due to settling and stratification of the heavy barite salts in the sand sample during transit.

As a check on recovery of barite barium by Fusion-XRF, we also conducted this analysis on the Barium Carbonate soil mixed 4:1 with pure barite. This gave a result of 11.55% barium, equal to 97% recovery.

The ALS Chemex Fusion-XRF method has an upper limit of approximately 50% barium. Pure barite is 59% barium by weight. Therefore this method should generate accurate total barium results up to a barite concentration of 85%. We did not verify the accuracy of the Fusion-ICP method at barium levels above 3,000 mg/kg, but it is presumed that this method could also measure barium up to 100% purity if appropriate dilutions are employed prior to ICP analysis.

5.2 Results for Strong Acid Leachable Barium Methods

Recovery of barium by acid digestion methods was significantly affected by soil texture and by lab-specific variables within the selected digestion techniques.

Roughly speaking, recovery of the acid digestion results versus the Fusion-XRF or Fusion-ICP results ranged from 23-75% for the spiked sand, from 23-70% for the spiked sandy clay loam and from 13-55% for the spiked clay. Recovery for the barium carbonate soil and the barite field soil by all acid digestion methods was consistently higher, ranging from approximately 60-90%

Recovery tended to be lowest for the BC SALM method as used by ALS in Vancouver, even compared to BC SALM analyses by NWL-EDM. ALS Vancouver follows the reference BC SALM method conditions, whereas NWL-EDM utilizes performance-based digestion conditions. Differences in barium recoveries are likely due to inter-laboratory differences in either the temperature of digestion, the duration of digestion, the soil to acid ratios utilized, or other factors not yet understood. These differences reinforce our initial concerns that seemingly small differences in laboratory methods can have significant impacts on strong acid leachable barium results (at least in the case of barite), and that these differences may not be identified during performance based validations of methods unless barite soils are specifically addressed. Until these factors are better understood and controlled, little reliance should be placed on the results of strong acid methods for barite barium.

It is important to note that US EPA 3050B contains two options; one uses nitric acid with peroxide, and the other employs nitric acid, peroxide, and hydrochloric acid. In addition to potential differences due to sample weights and dilution volumes used, these two alternative digestion techniques may also influence total barium recoveries. US EPA 3051 is another commonly used method for digestion of total metals, which is often used interchangeably with 3050B. The major difference between 3050B and 3051 is the use of a microwave digestion technique, rather than the hot block digestion technique of 3050B. It is commonly assumed that the techniques provide comparable digestions for metals, but the correctness of this assumption for barite barium is not understood.

Further investigation into differences between EPA 3050B Nitric/Peroxide, EPA 3050B Nitric/Peroxide/HCl, EPA 3051, and BC SALM is a recommended course of action for future studies concerning assessment of total barium techniques. Close attention must be paid within future studies to digestion temperatures, times, acid:solid ratios, and other potentially significant method variables.

Correlation of strong acid digestion barium recoveries versus true total barium concentration is also an important relationship to investigate, since it is predicted that all acid digestion methods will experience a concentration ceiling for total barium.

5.3 Results for Calcium Chloride Extraction Methods

A wide range of concentrations and several different extraction ratios were tested. Barium recovery was extremely sensitive to soil texture. For the spiked soils, using the standard Alberta Environment method with neutral salt solution at 0.1M, a 1:10 soil : solution ratio, and a 2 hour extraction period, only about 1 mg/kg barium was recovered from the clay, about 3 mg/kg from the sandy clay loam, and about 70 mg/kg from the sand.

This finding had not been anticipated, on the grounds that barite is insoluble and would essentially remain separate from the soil matrix, so that barium availability would depend mainly on barite solubility in the extracting solution. The effect of cation exchange capacity on recoveries of extractable barium from barite is greater than had been anticipated.

For the field soils, about 40 mg/kg of standard calcium chloride extractable barium was found in the "barite soil", versus 350 mg/kg in the "$BaCO_3$" soil, whereas both soils have fairly comparable total barium concentrations (2,700 vs. 2,460 mg/kg). This difference in soluble barium recovery is probably not due to texture, since both soils are medium-coarse. Given the site history of the "barium carbonate" test sample, the increased soluble barium is likely due to at least some presence of $BaCO_3$ in the sample, which is consistent with the history of the site (barite may also be present).

Changing the initial pH of the 0.1M calcium chloride extract (by using small amounts of strong acid or alkali) had little or no effect on barium recovery. This lack of change may be partially due to the fact that by the end of the shaking period, extract pH had returned to within 0.5 of a pH unit lower or higher than in a neutral extract, due to the buffering action of the soil being extracted. An acid or alkaline extract pH could be maintained for longer, by using strong solutions of weak acids or bases, or with buffers such as phthalate or borax; however, the extraction method would thus in effect be changed. However, results achieved to date, including results for the pH 4.93 TCLP extraction, indicate that reducing the initial pH of extracting solutions to pH 5 or even pH 3 has little to no effect on soluble barium.

Increasing the calcium chloride concentration from 0.05M to 1.0M greatly increased barium recovery, as did using a 2-fold wider soil:solution extraction ratio. At the 10:1 extraction ratio, the increase in calcium chloride concentration from 0.05M to 1.0M increased barium recovery from the barite spiked clay and sandy clay loam soils by a factor of 21 and 15 times, respectively. For the spiked sand sample, the increase in $CaCl_2$ concentration produced a lesser, but still significant increase of 3.6 times. This suggests that the increased $CaCl_2$ concentration is promoting increased recovery of barite in coarse textured soils by increasing its solubility through an ionic co-solvency effect. Barium recovery in fine textured soils appears to be increasing because of both an increased barite solubility and stronger cation exchange effects. The increasing solubility of barite with increasing concentrations of sodium chloride in water has been documented in the literature (Templeton, 1960).

A deionized water extraction of the barium carbonate soil (20:1 ratio) was conducted to assess the relative importance of cation exchange on the extraction methods. This produced the lowest results by far of any extraction method, with soluble barium of only 2 mg/kg, or approximately 100-200 times less recovery than with the more aggressive calcium chloride and EDTA methods.

5.4 Results for Chelation Methods

The Lindsay & Norvell DTPA extraction procedure and the SPLP procedure gave generally much lower recoveries than the calcium chloride methods. The concentration of DTPA in the Lindsay & Norvell method is quite low; also the chelated complex with barium is likely to be much less stable than complexes of the micronutrient metals (Fe, Cu etc.) for which this test is routinely used. The DTPA method as employed achieves far lower recoveries of soluble barium than all versions of the calcium chloride extraction method, likely because the 0.005M DTPA concentration was too low for this application.

Extraction using Na_2-EDTA solution gave results that agreed quite well with the standard calcium chloride method for all the soils in the study. The EDTA concentration was ten times higher than the DTPA concentration, which is likely a factor in the increased recoveries of the EDTA method relative to the DTPA method. Further increases in the EDTA concentration of this method may result in higher recoveries of barium. Because this method utilizes a 0.05M sodium salt of EDTA, there may also be cation exchange effects contributed from the presence of sodium.

Table 5. Comparison of All Soluble Barium Methods (Averages Shown)

Averages of Soluble Barium Methods	DI Water 20:1 Extr.	0.05 M CaCl2 10:1 Extr. pH=3	0.05 M CaCl2 10:1 Extr. pH=7	0.05 M CaCl2 10:1 Extr. pH=10	0.1M CaCl2 10:1 Extr. pH=7 AEnv	0.1M CaCl2 10:1 Extr. pH=3	0.1M CaCl2 20:1 Extr. pH=7	1.0M CaCl2 10:1 Extr. pH=7	1.0M CaCl2 20:1 Extr. pH=7	TCLP 20:1 Leach pH 4.93	SPLP 20:1 Leach	EDTA 0.05M 5:1 Extr.	DTPA 0.005 M 5:1 Extr.
BaCO3 soil	2.0				341	356	414	440	495	103	5.8	487	19.1
Spiked Clay		0.7	0.7	0.7	1.5		4.5	15	36	1.3	0.5	1.0	0.04
Spiked SCL		1.8	1.9	1.9	3.8		9.9	28	55	1.5	0.6	2.8	0.2
Spiked Sand		51	50	45	66		136	163	293	60	1.0	56	3.6
Barite Field Soil		21	20	20	38		67	106	189	43	0.60	9.8	0.8

5.5 Results for TCLP and SPLP Methods

The TCLP method also gave fairly similar recoveries to the Alberta Environment calcium chloride method. However, recoveries were very low for the clay and sandy clay loam soils. Without modification to incorporate a cation exchange process, these methods would not be expected to give reliable recoveries for soluble barium in fine-textured soils.

The SPLP method utilizes sulfuric acid in its extraction medium, which was immediately responsible for the precipitation of barium sulfate in all samples. For this reason, barium results in all samples were lowest by this method. SPLP was clearly not an appropriate technique for the determination of soluble barium.

5.6 Dilution Issues with ICP Analysis of Barium Digests / Extracts

Prior to barium analysis by ICP, sample extracts or digests may require dilution to within the linear range of the instrumentation. The degree of final dilution is dependent on the barium concentration as well as salinity, and on the actual instrument's upper limit of detection (usually higher for ICP-OES than ICP-MS). Because of the low solubility of barium, it is important to consider that final analytical results may potentially be influenced by variations in final solution volume. Large dilutions of the final digest may increase the measured barium concentration. This has not been investigated in detail, but should be considered when comparing test results among labs, and when designing analytical method schemes for general use.

6.0 RECOMMENDATIONS AND CONCLUSIONS

Refer to Figure 1 for a graphical comparison of most of the analytical results obtained for soluble barium methods. Methods involving pH change alone are excluded because they showed no effect.

The TCLP and SPLP methods can clearly be eliminated from further consideration. The SPLP method precipitates barium due to the presence of sulfuric acid, and the TCLP method does not adequately recover barium from cation exchange sites on fine textured soils.

Modifications to the pH of the calcium chloride extraction solutions produced no noticeable effect on barium recoveries of any test samples. Furthermore, the varied buffering potential of soil samples makes extraction pH a difficult variable to control. Achieving more effective pH buffering in a calcium chloride extraction method would require considerable further work and investigation, and is not merited given the effects observed thus far.

The standard 0.005M DTPA extraction was insufficiently vigorous for our purposes, with far less recoveries than any of the other techniques for all samples, including the relatively soluble form of barium from the barium carbonate soil. A significantly increased DTPA concentration may improve its performance, but the data available indicates that the EDTA chelation method would likely perform as well or better. No additional studies are recommended for this method.

The standard EDTA method performed similarly to the Alberta Environment calcium chloride method, and may have potential as a viable method for soluble barium. However, the mechanism of this extraction technique for barium is less understood than other techniques, particularly in terms of the chelation of barium, and how this may be affected by uncontrolled variables. To further pursue this method, considerably more investigation would be required into the effects of extraction ratio and EDTA concentration on barite recoveries, and on other factors that may affect chelation. We recommend that no further work is done on EDTA methods, unless the calcium chloride methods cannot meet the objectives of BC MOE. The calcium chloride methods are better understood, are known to be rugged and easy to use, and will give some consistency with the approach used in Alberta.

Increasing the calcium chloride extract concentration from 0.1 M to 1.0 M, combined with widening the extraction ratio from 10:1 to 20:1 were very effective in increasing the recoveries of barium from spiked and real-world soils. Optimization of these variables may be environmentally relevant for two reasons:

i) Increased $CaCl_2$ concentration increases the recovery of barium from cation exchange sites on fine textured samples. Barium adsorbed to cation exchange sites may be environmentally available, as it is unlikely to be in the form of barite.

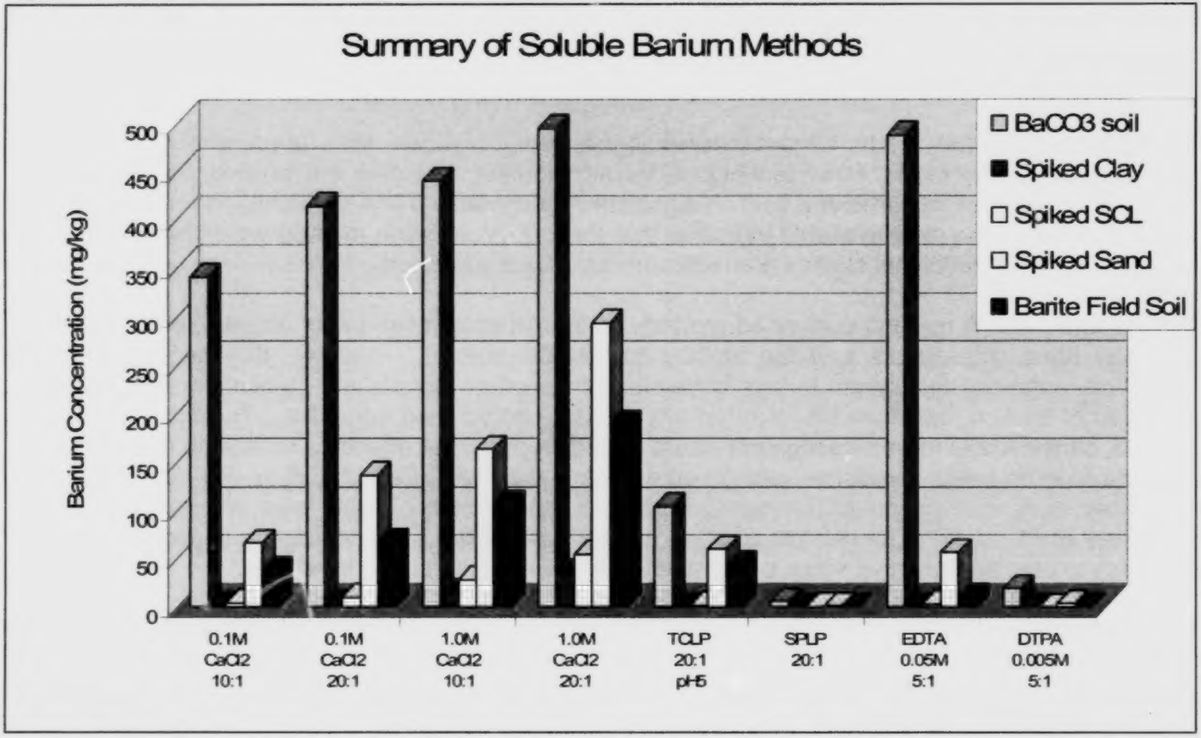
ii) Increased $CaCl_2$ concentrations, which appear to increase the solubility of barite in laboratory solutions, could simulate the effect of brines from produced water that may be present in some drilling waste sites (Note however that brine solutions at drilling waste sites would likely never be found with salt concentrations as high as 1.0M).

Modifications to $CaCl_2$ concentration and extraction ratio would be simple and effective, yet the extraction method remains essentially the same as the standard Alberta Environment method for soluble barium. Inclusion of one or both of these modifications would seem to be the obvious changes to make the method more aggressive in terms of the recovery of soluble barium from fine-textured, retentive soils. These changes were much more effective than leaving the concentration of $CaCl_2$ unchanged at 0.01, and lengthening the extraction time.

However, soluble barium recoveries of these methods must first be evaluated using coarse textured soils with higher concentrations of barite (as would actually be encountered at drilling waste sites), to determine whether there is an upper ceiling for soluble barium by these techniques, and what it may be. The optimum method should not be overly aggressive for the liberation of barite.

Any optimized method should also be more extensively evaluated with more soluble forms of barium than barite (e.g. barium chloride), which would likely be necessary to establish a CSR standard for soluble barium by the method.

Figure 1. Graphical Comparison of Selected Soluble Barium Methods



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Appendix 1. Detailed Characterization of Background Soils

Method		Parameter Name	Unit	Detection Limit	Sand	Clay	Silty Clay Loam
Cation Exchange Capacity (CEC) for General Soil		Calcium	mg/kg	4	1970	7720	8130
		Magnesium	mg/kg	2	34	1910	301
		Potassium	mg/kg	20	<20	260	80
		Sodium	mg/kg	12	<10	213	30
		Base saturation	%	1	780	227	345
		Calcium	meq/100g	0.0003	9.81	38.5	40.6
		Magnesium	meq/100g	0.0008	0.28	15.7	2.48
		Sodium	meq/100g	0.003	<0.05	0.925	0.13
		Potassium	meq/100g	0.003	<0.05	0.68	0.2
		ESP	%	0.2	<4	3.76	1
		TEC	meq/100g	2	10	56	43
		CEC	meq/100g		1.3	24.6	12.6
Carbon and Nitrogen in soil (FSJ)		Organic Matter	%	1	<1	2	<1
		Carbon	%	0.05	<0.05	1.13	0.43
Hot Water Soluble		Boron	ug/g	0.1	<0.1	1	0.7
Metals Strong Acid Digestion	Mercury (Hot Block) in Soil	Mercury	ug/g	0.01	0.01	0.08	0.04
	Metals ICP-MS (Hot Block) in soil	Antimony	ug/g	0.2	<0.1	<0.2	<0.2
		Arsenic	ug/g	0.2	2.4	10.5	8.4
		Barium	ug/g	1	64	333	195
		Beryllium	ug/g	0.1	0.1	1.2	0.7
		Cadmium	ug/g	0.01	0.06	0.42	0.28
		Chromium	ug/g	0.5	3.4	34.4	21.4
		Cobalt	ug/g	0.1	2.2	12.8	10.7
		Copper	ug/g	1	3	32	19
		Lead	ug/g	0.1	2.3	14.1	9.9
		Molybdenum	ug/g	1	<1	<1	<1
		Nickel	ug/g	0.5	6.1	38.3	29.6
		Selenium	ug/g	0.3	<0.2	1.4	0.4
		Silver	ug/g	0.1	<0.1	0.2	0.2
		Thallium	ug/g	0.05	0.06	0.3	0.26
		Tin	ug/g	1	1	<1	<1
		Vanadium	ug/g	0.1	6	65.4	43.5
		Zinc	ug/g	1	12	111	65

Appendix 1. Detailed Characterization of Background Soils (cont'd).

Method		Parameter Name	Unit	Detection Limit	Sand	Clay	Silty Clay Loam
Physical and Aggregate Properties	Moisture	Moisture	%	0.1	1.6	23	12.1
	Particle Size Analysis - GS	Texture			Sand	Clay	Sandy Clay Loam
		Sand	%		96.4	14.4	45.7
		Silt	%		1.2	26.2	25.3
		Clay	%		2.4	59.4	29
Salinity	Saturated Paste in General Soil	pH	pH		8.4	7.7	7.8
		EC	dS/m	0.01	0.21	4.05	2.53
		SAR			0.2	1	0.2
		% Saturation	%		35	108	55
		Calcium	meq/L	0.01	1.89	26.9	32.5
		Calcium	mg/kg		13.1	583	358
		Magnesium	meq/L	0.02	0.38	36.9	7.43
		Magnesium	mg/kg		1.6	484	49.5
		Sodium	meq/L	0.04	0.19	5.89	0.9
		Sodium	mg/kg		2	147	11
		Potassium	meq/L	0.03	0.06	0.69	<0.2
		Potassium	mg/kg		<1	29	<6
		Chloride	meq/L	0.03	0.07	1.31	0.14
		Chloride	mg/kg		1	50	3
		Sulfate-S	meq/L	0.06	0.61	64.9	37
		Sulfate-S	mg/kg		3.4	1120	326
		TGR	T/ac		<0.1	<0.1	<0.1
$CaCl_2$ Extractable Barium			mg/kg	0.05	9.4	1.1	2.7

Appendix II

Dodd et al. (2007) –
Barite Bioaccessibility Report

BARITE BIOACCESSIBILITY STUDY

Project Prepared for:

Canadian Association of Petroleum Producers
Suite 2100, 350 7th Avenue SW
Calgary, Alberta T2P 3N9

Prepared by:

Matt Dodd

Royal Roads University
2005 Sooke Road, Victoria, B.C., V9B 5Y2
Canada

Yvette Lowney

Exponent Inc.
4940 Pearl East Circle, Boulder, CO 80301
USA

and

Miles Tindal

Axiom Environmental Inc.
438 Eagle Heights, Canmore, AB T1W 3C9
Canada

July 4, 2007

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Appendix B Exponent Bioaccessibility Report

EXECUTIVE SUMMARY

INTRODUCTION

Barite (barium sulphate) is a ubiquitous component of oilfield drilling muds, and commonly remains on wellsites after drilling operations are complete. Subsequent environmental investigations of soil quality at such sites often identify total barium at concentrations above the applicable barium guideline value. However barium guidelines are based on soluble barium compounds while barite is highly insoluble. Accordingly, some jurisdictions (Alberta, British Columbia) have developed or are developing soil quality guidelines applicable to barite. Currently, in the absence of information to the contrary in the published literature, barite soil quality guidelines have assumed that barite is 100% bioavailable in the mammalian gut. This study uses in vitro bioaccessibility techniques to make a conservative estimate of the bioavailability of barite in the mammalian gut.

In recent years, chemical extraction techniques have been developed that mimic the conditions (pH, temperature, agitation, enzymes, residence time, etc) in the mammalian stomach and/or small intestine. The amount of a chemical solubilized in these extractions is referred to as the bioaccessible fraction of the chemical. While not all the bioaccessible fraction of a chemical may necessarily be absorbed into the body, it is generally assumed that dissolution in the gut is a necessary precursor to absorption, and thus *in vitro* measurements of bioaccessibility are a practical method to obtain reasonable, conservative estimates of bioavailability.

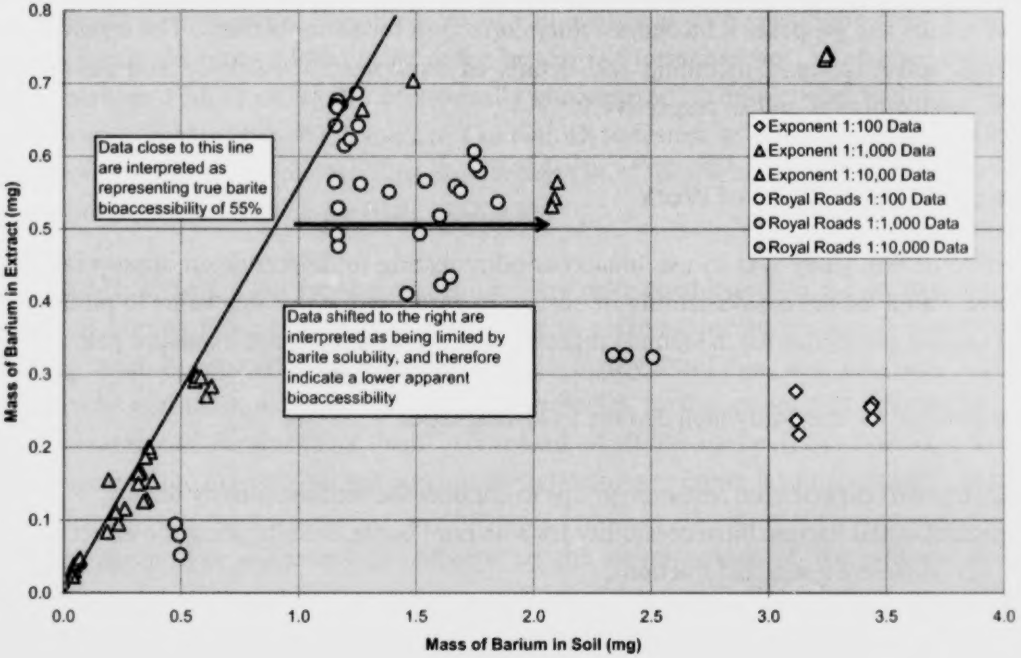
METHODOLOGY

The use of bioaccessibility techniques to make bioavailability corrections in soil quality guidelines in Canada is relatively new. Accordingly two research groups (from Exponent Inc., and Royal Roads University) were contracted to work independently on this project, so that any possible bias from the techniques used in a particular lab could be removed. Bioaccessibility extractions were conducted using standard published methodologies. Initial extractions were completed using pure barite and investigated the effect of grain size, pH, and fluid:solid extraction ratios. A second phase of work involved conducting extractions on five barite-contaminated field soils using various grain size fractions and extraction ratios. In all, over one hundred extractions were completed on these five soils.

RESULTS AND CONCLUSIONS

Bioaccessibility is defined as the mass of chemical in the extract divided by the original mass of chemical in the soil/solid. Accordingly, Figure A presents a graph of the mass of barium in the extract vs. the mass of barium in the soil. Many of the data points lie close to or just below the line indicated on the Figure. This line corresponds to a bioaccessibility of 55%, and the points lying close to this line are interpreted as being consistent with a bioaccessibility of 55%. Other points on Figure A are shifted to the right of this line. This behavior is interpreted as indicating test conditions where bioaccessibility is being limited by the solubility of barite in the extracting solution. No points on Figure 2 lie significantly to the left of the black line, and thus a barite bioaccessibility of 55% appears to be conservative over a range of sites, extraction conditions, and grain sizes.

Figure A. Barium Extraction Data Interpreted as Bioaccessibility



Bioaccessibility measurements are considered to be a reasonable conservative estimate of the oral bioavailability of a chemical. Extensive work completed for this study indicates that 55% is a conservative estimate of the *bioaccessibility* of barium in barite-contaminated soils. Accordingly, this report concludes that 55% is a reasonable, conservative estimate of the oral *bioavailability* of barite in soil for use in the calculation of soil quality guidelines protective of mammalian soil ingestion.

1. INTRODUCTION

The Canadian Association of Petroleum Producers retained Axiom Environmental Inc. to commission work in support of developing a bioavailability adjustment for the Alberta Environment soil quality guideline for barite. Bioaccessibility lab studies were contracted to Yvette Lowney, of Exponent Inc., Boulder, Colorado, USA and Matt Dodd of Royal Roads University. This report provides background information relevant to the study, a summary and analysis of results and proposes a bioaccessibility correction for barite barium. The reports from Royal Roads and Exponent, including full details of experimental methods and results are provided in Appendices A and B, respectively.

1.1 Objectives and Scope of Work

The objective of this study was to use bioaccessibility testing to determine an appropriate, but conservative, value for the bioavailability of barium in barite and to use this value to modify the barite soil quality guidelines for the human ingestion and wildlife ingestion exposure pathways.

The scope of work for this study included the following tasks:

- Identify two experienced research groups to conduct the bioaccessibility testing.
- Conduct initial barium bioaccessibility tests on pure barite, investigating the effect of:
  a) different grain size fractions;
  b) varying the pH of the extracting solution; and,
  c) different fluid:solid extraction ratios.
- Conduct barium bioaccessibility tests on up to five barite-contaminated field soils, investigating the effect of:
  d) different grain size fractions; and,
  e) different fluid:solid extraction ratios.
- Assess and analyze the results of the study, and, if appropriate, make a recommendation for a bioavailability correction to be used to modify the human and wildlife soil ingestion guidelines for barite.
- Generate a report on the study findings.

2. BACKGROUND

2.1 Problem Statement

Barite (barium sulphate) is a ubiquitous component of oilfield drilling muds, and commonly remains on wellsites after drilling operations are complete. Depending on composition, used drilling mud may be disposed of on-site using "mix-bury-cover" or other techniques. Subsequent environmental investigations of soil quality at such sites often identify total barium at concentrations up to 3,000 mg/kg in the location of former sumps, and higher concentrations, up to perhaps 10,000 mg/kg are occasionally encountered. These concentrations of barium are significantly higher than the Canadian Council of Ministers of the Environment (CCME) and British Columbia Contaminated Sites Regulation (BC CSR) Schedule 4 guidelines for barium in agricultural land use, which are both 750 mg/kg.

The CCME and BC CSR barium guidelines were developed based on the ecotoxicity of soluble forms of barium (e.g., barium chloride) to plants and soil invertebrates. However, barium in drilling mud is sourced from barite, which is highly insoluble, and has been shown to be effectively non-toxic to plants and soil invertebrates to the extent that plants can grow and invertebrates can survive in a "soil" composed of 100% barite (ESG, 2003). Accordingly, application of the existing barium soil quality guidelines to barite is unnecessarily conservative.

This situation was addressed in Alberta by the development of the *Alberta Soil Quality Guidelines for Barite* (AENV, 2004; see Section 2.2 below). This project considered all the potential exposure pathways through which barite in soil could reach receptors of concern. The limiting pathways were human soil ingestion (agricultural, residential, commercial and industrial land use) and wildlife soil ingestion (natural area land use). The guidelines for both these pathways assumed that barite was 100% bioavailable in the mammalian digestive tract, since no published data were available to support a compound-specific bioavailability for barite. This study uses bioaccessibility testing to develop a conservative value of the mammalian bioavailability of barite.

2.2 Existing Barite Guideline Initiatives

As noted above, Alberta Environment developed the Alberta Soil Quality Guidelines for Barite, available at: http://environment.gov.ab.ca/info/library/6298.pdf. The Alberta Guideline is implemented in two steps. In the first step the "extractable barium" is measured, as an indication of the amount of barium that is biologically available to plants and soil invertebrates. For Natural Area or Agricultural land use, if the *extractable* barium is less than 260 mg/kg, then the barium at the site is determined to be in a sufficiently unavailable form to be considered as barite, and the proponent can proceed to the second step, and assess the site based on a barite guideline of 3,300 mg/kg (Natural Area) or 3,200 mg/kg (Agricultural Land) which applies to *total* barium. The development of the Alberta Barite Guideline carefully considered all aspects of the environmental fate and transport of barium, including the potential for barium mobilization via sulphate reduction. The Alberta Barite Guideline document concluded that barite in buried oilfield sumps was likely to remain in the form of barite indefinitely, and that the barium was unlikely to be mobilized by any environmental processes that could reasonably be foreseen.

There in an ongoing initiative in British Columbia to implement a Schedule 5 Matrix Soil Standard for barite-barium. Much of the background science that was used to develop the Alberta Barite Soil Quality Guidelines will also be used in the BC standard, but the implementation will be somewhat different, reflecting the different legislative basis for contaminated site management in that jurisdiction.

2.3 Bioaccessibility Testing

2.3.1 Scientific Principle

When an inorganic chemical is ingested, a fraction of the ingested chemical may be absorbed through the gastro-intestinal (GI) tract, and enter the internal systems of the body. This internalized part of the chemical is referred to as the bioavailable fraction. Traditionally, the oral bioavailability of a chemical has been determined through testing using an appropriate animal model. Examining this process in a little more detail allows the absorption of ingested chemicals to be split into two steps. In the first step, the chemical is dissolved in the GI tract. The proportion of the chemical that is dissolved is referred to as the bioaccessible fraction. The second step is the absorption of all or part of the bioaccessible fraction.

Definitive bioavailability studies are complex and expensive. In recent years, techniques have been developed (Ruby et al., 1993, 1996, 1999; Ruby 2004; Medlin, 1997; and Rodriguez et al., 1999) that mimic the conditions (pH, temperature, agitation, enzymes, residence time, etc) in the stomach and/or small intestine to measure the bioaccessible fraction of a chemical *in vitro*. While not all the bioaccessible fraction of a chemical may necessarily be absorbed, it is generally assumed (e.g., Ruby et al., 1999) that dissolution in the GI tract is a necessary precursor to absorption, and thus *in vitro* measurements of bioaccessibility are a practical method to obtain reasonable, conservative estimates of bioavailability.

2.3.2 Rationale for Use in this Study

As noted in Section 2.3.1 above, there is confidence that measured bioaccessibility for an inorganic chemical is similar to or greater than actual bioavailability, and therefore bioaccessibility is an appropriate and conservative technique for estimating a bioavailability correction in the development of soil quality guidelines for barite.

2.3.3 Health Canada Concerns Addressed

Health Canada (Richardson et al., 2006) considers that bioaccessibility techniques are useful and appropriate methods for estimating bioavailability corrections for human health risk assessment in Canada, but emphasizes two main points that must be considered in the study design. The first is that either physiologically relevant (order of 10,000:1) solution:solid extraction ratios are used or it is otherwise determined that the bioaccessibility values measured are not being limited by the solubility of the compound in question.

The second concern is that the physiologically relevant grain size for incidental soil ingestion is small (perhaps as low as 10 μm). Thus it is important to have a good understanding of how bioaccessibility may change with grain size. Both these concerns are addressed in the experimental design (Section 3.2) for this study.

2.4 Challenges with Measuring Total Barium in Barite Samples

Conventional analytical methods for determining total metals in environmental soil samples include US EPA methods 3050, 3050B, 3051, and, in British Columbia the Strong Acid Leachate Method (SALM). All of these methods involve extracting the soil using a strong acid at relatively low solution:solid extraction ratios (5:1 or 10:1). These methods have typically been found to give acceptable performance for most metals of environmental concern. However, ongoing work into analytical techniques for total and soluble barium being conducted on behalf of the British Columbia Ministry of the Environment (BCE) has suggested that these techniques do not provide adequate recovery of barium from soils contaminated with higher concentrations of barite. The issue appears to be in getting quantitative extraction of barium into the aqueous phase. Once the barium is in the aqueous phase, any of the standard analytical techniques (e.g., ICP, OES) appear to be satisfactory for quantifying barium in an aqueous extract.

When conducting the bioaccessibility work in this study, it was important to know the true total amount of barium in the soil sample, so that a meaningful bioaccessibility value could be calculated. Subsequent to starting the current study, the BCE work referenced above found that sample fusion followed by x-ray fluorescence (Fusion-XRF) was an effective technique to measure total barite-barium, achieving effectively quantitative recovery. Accordingly, Fusion-XRF analyses were conducted on samples from each of the sites after the bioaccessibility work had been completed, and the bioaccessibility values reported in Tables 3 and 4, and in Figure 1, Figure 2, and Figure A were calculated based on the XRF analysis of the concentration of the barium in the soil samples (i.e., the true total barium concentration).

There is no corresponding concern with the analytical technique used to evaluate the barium concentration in the extracts used for the bioaccessibility testing. As noted above, any of the standard analytical techniques appear to be satisfactory for quantifying barium in an aqueous extract.

2.5 Extraction Ratio Naming Conventions

It is noted that two different naming conventions for extraction ratios are used by the two research groups. Royal Roads (Appendix A) uses the fluid:soil ratio (e.g., 10,000:1), and hence a larger ratio corresponds to a more dilute extraction. This is also the convention used in the main text of this report, and is consistent with Richardson et al. (2006). Exponent (Appendix B) uses the opposite convention, quoting the soil:fluid ratio (e.g., 1:10,000).

3. METHODOLOGY

3.1 Methodology for Bioaccessibility Work

The Royal Roads study (Appendix A) included initial experiments to compare the effectiveness of two possible digestion models for use with barite. The first was the Simplified Bioaccessibility Extraction Test (SBET) which is a 1 hour extraction simulating conditions in a mammalian stomach. The second method was the Sequential Stomach and Small Intestine Phase Extraction model, which conducts a 1 hour extraction under conditions mimicking a mammalian stomach, followed by a 3 hour extraction under conditions mimicking a mammalian small intestine. Both these methods are sourced from Battelle and Exponent (2000), which is perhaps the definitive review on such techniques.

The Royal Roads work indicated that the two methods gave similar results for barite, and that the simpler SBET method actually appeared to be slightly the more conservative of the two methods. The SBET methodology was used by both Royal Roads and Exponent for the remainder of the study. Full details of the methodology used by Royal Roads and Exponent are provided in Appendices A and B.

3.2 Study Design

As noted previously, two research groups were contracted to conduct the bioaccessibility work for this project. Each group has unique and valuable expertise in this field which it was felt would be a major contribution to the project. In addition, using bioaccessibility testing to make bioavailability corrections in soil quality guideline development is a new field in Canada, and it was considered that the additional corroboration from having two independent labs work on the same project would be valuable. The research group at Exponent Inc., in Boulder, Colorado, USA, headed by Yvette Lowney, have been some of the major proponents and developers of physiologically based extraction techniques (PBETs) since the first work in this field in the early 1990s. The Royal Roads University group, headed by Matt Dodd, has worked closely with Health Canada on the implementation of bioaccessibility in human health risk assessment in Canada.

The study design for this work involved two main steps.

The first step (designated Phase 1 and 2, for historical reasons) involved a series of experiments working with pure barite, and investigated the effects on barium bioaccessibility of changing grain size, fluid:solid extraction ratio, and pH. Some work was also done to assess an appropriate digestion model.

The second step ("Phase 3") involved a series of extractions with field-contaminated soils containing barite. Soils from five sites (designated Site #1 to Site #5) were used. Due to limited soil volume available in some cases, Exponent had Site #1 to Site #4 available, while Royal Roads had access to Site #2 to Site #5. For each Site, extractions were completed using a range of different extraction ratios and grain sizes.

The number of repetitions for each extraction completed by each of the groups is summarized in Tables 1 and 2. Full details of the experimental design for each study are provided in Appendices A and B.

3.3 Experimental Soils

Five experimental soils from three different areas of Alberta were obtained for analysis in this project. All the soils were from drilling waste treatment sites, and were donated by the companies involved. All of the sites came from the Alberta/BC Rocky Mountain Foothills, since sites with higher barite concentrations were being selected, and typically, more barite is required to drill wells in the foothills region, due to the higher downhole pressures.

- Site 1 was a coarse-grained soil from near Grand Prairie, Northwest Alberta

4. RESULTS – SUMMARY AND DISCUSSION

Full details of the results of each study are provided in Appendices A and B. Key results for the barite contaminated field soils (Phase 3) are provided in Tables 3 and 4. A discussion of results follows.

4.1 Phase 1 and 2

Phase 1 and 2 involved an investigation of the effect on barium bioaccessibility of changing grain size, fluid:solid extraction ratio, and pH. Results from the Royal Roads and Exponent work agreed with each other and confirmed the following points:

- Bioaccessibility increases at lower pH values. Accordingly, a conservative low pH value of 1.5 was used for the studies.
- The measurement of barium bioaccessibility from barite can be limited by the solubility of barite in the extracting solution. Bioaccessibility therefore increases at higher fluid:solid extraction ratios. This factor was taken into consideration in the analysis of the Phase 3 results (see Section 4.2 below).
- Grain size did not appear to have a major effect on bioaccessibility. The variation that was seen can likely be explained by analytical variation.

4.2 Phase 3

The results of the Phase 3 studies with barite-contaminated field soils are summarized in Tables 3 (Exponent data) and 4 (Royal Roads data). Full details are available in Appendices A and B.

Bioaccessibility is defined as the mass of chemical in the extract divided by the original mass of chemical in the soil/solid. Accordingly, Figure 1 presents a graph of the mass of barium in the extract vs. the mass of barium in the soil. Based on the discussion in Section 2.4, the mass of barium in soil is calculated from the Fusion-XRF analysis. Figure 1 shows all the Phase 3 data for both research providers, for the five sites, for all dilution ratios and grain sizes, and all available repetitions. Figure 2 shows the same data on an expanded scale to allow some of the relationships to be seen more clearly.

In Figure 2, it can be seen that many of the data points lie close to or just below the line indicated in black. This line indicates a bioaccessibility of 55%, and the points lying close to this line are interpreted as being consistent with a bioaccessibility of 55%. (Based on the definition of bioaccessibility provided above, all data consistent with a particular bioaccessibility would be expected to lie on a straight line passing through the origin, with the slope of the line indicating the bioaccessibility.)

Other points on Figure 2 (and Figure 1) are shifted to the right of this line. This behavior is interpreted as indicating test conditions where bioaccessibility is being limited by the solubility of barite in the extracting solution (if solubility is limiting, then increasing the mass of barium in the soil does not increase the mass of barium in the extract, and points are shifted to the right, corresponding to lower apparent bioaccessibility).

No points on Figure 2 lie significantly to the left of the black line, and thus a barite bioaccessibility of 55% appears to be conservative over a range of sites, extraction conditions, and grain sizes.

5. CONCLUSION

As noted in Section 2.3.1, bioaccessibility measurements are considered to be a reasonable conservative estimate of the oral bioavailability of a chemical. Extensive work completed for this study and including over 100 physiologically-based extractions of five different barite-contaminated field soils indicates that 55% is a conservative estimate of the *bioaccessibility* of barium in barite-contaminated soils. Accordingly, this report concludes that 55% is a reasonable, conservative estimate of the oral *bioavailability* of barite in soil for use in the calculation of soil quality guidelines protective of mammalian soil ingestion.

6. CLOSURE

The information presented in this report was compiled and interpreted exclusively for the purposes stated in Section 1.1 of the document. Axiom Environmental Inc. (Axiom) provided this report for the Canadian Association of Petroleum Producers (CAPP) solely for the purpose noted above.

Axiom has exercised reasonable skill, care and diligence to assess the information acquired during the preparation of this report, but makes no guarantees or warranties as to the accuracy or completeness of this information. The information contained in this report is based upon, and limited by, the circumstances and conditions acknowledged herein, and upon information available at the time of its preparation. The information provided by others is believed to be accurate but cannot be guaranteed.

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Any questions concerning the information in this report or its interpretation should be directed to Miles Tindal at (403) 678 4790, or mtindal@axiomenvironmental.ca.

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Table 1. Experimental Design - Exponent

Test or Soil ID	Fluid: Soil Ratio	Number of Reps for Each Grain Size				
Tests with Pure Barite (Phase 1 and 2)						
		pH				
		1.5	**4.5**			
pH Test	1,000:1	3	3			
		Fluid:Solid Ratio				
		100:1	**300:1**	**1,000:1**	**3,000:1**	**10,000:1**
Extraction Ratio Tes	various	5	4	4	4	3
		Grain Size (μm)				
		45-325	**180-250**	**<38**		
Grain Size Test	1,000:1	3	3	3		
Tests with Field Contaminated Soil (Phase 3)						
		Grain Size (μm)				
		<2000	**<250**	**<38**		
Site #1 Test	100:1	3	3	3		
Site #1 Test	1,000:1	3	3	3		
Site #1 Test	10,000:1	3	3	3		
Site #2 Test	100:1	3	3	3		
Site #2 Test	1,000:1	3	3	3		
Site #2 Test	10,000:1	3	3	3		
Site #3 Test	100:1	-	-	3		
Site #3 Test	1,000:1	-	-	1		
Site #3 Test	10,000:1	-	-	1		
Site #4 Test	100:1	-	-	3		
Site #4 Test	1,000:1	-	-	1		
Site #4 Test	10,000:1	-	-	1		
Site #1 Control	100:1	3	3	3		
Site #1 Control	1,000:1	3	3	3		
Site #1 Control	10,000:1	3	3	3		

Table 2. Experimental Design - Royal Roads

Test or Soil ID	Fluid: Soil Ratio	Number of Reps					
Tests with Pure Barite (Phase 1 and 2)							
		Grain Size (µm)					
Digestion Model Test		**<250**	**<125**	**<63**	**<45**		
SBET Extraction	1,000:1	3	3	3	3		
Sequential Extraction [a]	1,000:1	3	3	3	3		
		pH					
		1.1	**1.5**	**2.0**	**3.0**	**4.0**	
pH Test	1,000:1	3	3	3	3	3	
		Fluid:Solid Ratio					
		100:1	**500:1**	**1,000:1**	**2,000:1**	**5,000:1**	**10,000:1**
Extraction Ratio Test	various	3	3	3	3	3	3
		Grain Size (µm)					
		<250	**<125**	**<63**	**<45**		
Grain Size Test	1,000:1	3	3	3	3		
OECD Soil Tests							
Spiked with Barite [b]	1,000:1	3					
Control	1,000:1	1					
Tests with Field Contaminated Soil (Phase 3)							
		Grain Size (µm)					
		<250	**<125**	**<63**	**<45**		
Site #2 Test	100:1	-	-	-	3		
Site #2 Test	1,000:1	-	-	-	3		
Site #2 Test	10,000:1	-	-	-	3		
Site #2 Control	1,000:1	3	-	-	-		
Site #3 Test	100:1	-	-	-	3		
Site #3 Test	1,000:1	3	3	3	3		
Site #3 Test	10,000:1	-	-	-	3		
Site #3 Control	1,000:1	3	-	-	-		
Site #4 Test	100:1	-	-	-	3		
Site #4 Test	1,000:1	3	3	3	3		
Site #4 Test	10,000:1	-	-	-	3		
Site #5 Test	100:1	-	-	-	3		
Site #5 Test	1,000:1	-	-	-	3		
Site #5 Test	10,000:1	-	-	-	3		

Notes:

a. "Stomach" followed by "small intestine" extraction. See text and Appendix A for details.

b. Spiked with barite at 1 g per 50 g soil (11,770 mg/kg barium)

Table 3. Properties of Experimental Soils

		Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**
Location		Grande Prairie	Ricinus	Cutbank 5-26	Cutbank 9-24	Cutbank 11-7
Region		Northwest Alberta	Central Alberta Foothills	NE BC/Alberta Border	NE BC/Alberta Border	NE BC/Alberta Border
"Total" barium Fusion/XRF	mg/kg	5,328	56,732	11,537	14,501	-
"Total" barium by EPA 3050	mg/kg	5,084	8,737	9,185	6,875	1,443
Extractable barium by CaCl	mg/kg	6	49	50	35	54
Chloride	mg/L	35	100	-	-	-
Sulphate	mg/L	1,720	1,100	-	-	-
pH	-	7.7	7.3	-	-	-
EC	dS/m	2.77	16.6	-	-	-
MUST PSA >75μm	%	-	-	24	19	-
Particle Size - D10	μm	6.04	1.12	0.81	0.95	-
Particle Size - D50	μm	209	6.01	2.1	3.03	-
Particle Size - D90	μm	1,004	44.3	6.59	19.03	-
Soil texture classification		coarse	fine	fine	fine	fine

Notes:

a. Exponent Data for <38um fraction

b. Exponent Data for <38um fraction except for Site 5, Royal Roads data for whole sample

c. Analyzed on whole sample using Alberta 0.1M $CaCl_2$ method for soluble barium.

Table 4. Bioaccessibility Data Summary - Exponent

Soil ID	Grain Size	Barium Conc. in Soil [a] (mg/kg)	Mass of Soil Tested (g)	Mass of Barium in Soil Extracted [a] (mg)	Barium Conc. in Extract [b] (mg/L)	Volume of Extract (L)	Mass of Barium in Extract (mg)
Solid to Fluid Ratio = 1:100							
Site #1 Test	<2 mm	3,104	1.0078	3.128	2.178	0.100	0.218
Site #1 Test	<2 mm	3,104	1.0040	3.116	2.770	0.100	0.277
Site #1 Test	<2 mm	3,104	1.0046	3.118	2.376	0.100	0.238
Site #1 Test	<250 µm	3,418	1.0075	3.444	2.393	0.100	0.239
Site #1 Test	<250 µm	3,418	1.0069	3.442	2.605	0.100	0.261
Site #1 Test	<250 µm	3,418	1.0046	3.434	2.555	0.100	0.256
Site #1 Test	<38 µm	5,328	1.0011	5.334	2.271	0.100	0.227
Site #1 Test	<38 µm	5,328	1.0076	5.368	2.207	0.100	0.221
Site #1 Test	<38 µm	5,328	1.0058	5.359	2.236	0.100	0.224
Site #2 Test	<2 mm	20,762	1.0009	20.780	8.753	0.100	0.875
Site #2 Test	<2 mm	20,762	1.0012	20.786	8.692	0.100	0.869
Site #2 Test	<2 mm	20,762	1.0014	20.791	8.561	0.100	0.856
Site #2 Test	<250 µm	32,175	1.0064	32.381	7.834	0.100	0.783
Site #2 Test	<250 µm	32,175	1.0018	32.233	7.998	0.100	0.800
Site #2 Test	<250 µm	32,175	1.0013	32.217	8.001	0.100	0.800
Site #2 Test	<38 µm	56,732	1.0023	56.863	7.052	0.100	0.705
Site #2 Test	<38 µm	56,732	1.0000	56.732	6.950	0.100	0.695
Site #2 Test	<38 µm	56,732	1.0011	56.795	6.901	0.100	0.690
Site #3	<38 µm	11,537	1.0135	11.693	11.120	0.100	1.112
Site #3	<38 µm	11,537	1.0124	11.680	11.050	0.100	1.105
Site #3	<38 µm	11,537	1.0036	11.578	11.200	0.100	1.120
Site #4	<38 µm	14,501	1.0219	14.818	8.300	0.100	0.830
Site #4	<38 µm	14,501	1.0121	14.677	8.350	0.100	0.835
Solid to Fluid Ratio = 1:1,000							
Site #1 Test	<2 mm	3,104	0.1057	0.328	1.664	0.100	0.166
Site #1 Test	<2 mm	3,104	0.1027	0.319	1.638	0.100	0.164
Site #1 Test	<2 mm	3,104	0.1028	0.319	1.499	0.100	0.150
Site #1 Test	<250 µm	3,418	0.1077	0.368	1.935	0.100	0.194
Site #1 Test	<250 µm	3,418	0.1060	0.362	1.998	0.100	0.200
Site #1 Test	<250 µm	3,418	0.1015	0.347	1.864	0.100	0.186
Site #1 Test	<38 µm	5,328	0.1050	0.559	2.912	0.100	0.291
Site #1 Test	<38 µm	5,328	0.1053	0.561	3.003	0.100	0.300
Site #1 Test	<38 µm	5,328	0.1062	0.566	2.977	0.100	0.298
Site #2 Test	<2 mm	20,762	0.1006	2.089	5.431	0.100	0.543
Site #2 Test	<2 mm	20,762	0.1001	2.078	5.315	0.100	0.532
Site #2 Test	<2 mm	20,762	0.1012	2.101	5.649	0.100	0.565
Site #2 Test	<250 µm	32,175	0.1011	3.253	7.425	0.100	0.743
Site #2 Test	<250 µm	32,175	0.1007	3.240	7.333	0.100	0.733
Site #2 Test	<250 µm	32,175	0.1009	3.246	7.393	0.100	0.739
Site #2 Test	<38 µm	56,732	0.1024	5.809	8.739	0.100	0.874
Site #2 Test	<38 µm	56,732	0.1003	5.690	8.646	0.100	0.865
Site #2 Test	<38 µm	56,732	0.1007	5.713	8.689	0.100	0.869
Site #3	<38 µm	11,537	0.1100	1.270	6.644	0.100	0.664
Site #4	<38 µm	14,501	0.1025	1.487	7.042	0.100	0.704

Table 4. Bioaccessibility Data Summary - Exponent

Soil ID	Grain Size	Barium Conc. in Soil[a] (mg/kg)	Mass of Soil Tested (g)	Mass of Barium in Soil Extracted[a] (mg)	Barium Conc. in Extract[b] (mg/L)	Volume of Extract (L)	Mass of Barium in Extract (mg)
Solid to Fluid Ratio = 1:10,000							
Site #1 Test	<2 mm	3,104	0.0123	0.038	0.307	0.100	0.031
Site #1 Test	<2 mm	3,104	0.0143	0.044	0.303	0.100	0.030
Site #1 Test	<2 mm	3,104	0.0134	0.042	0.225	0.100	0.023
Site #1 Test	<250 µm	3,418	0.0115	0.039	0.227	0.100	0.023
Site #1 Test	<250 µm	3,418	0.0140	0.048	0.382	0.100	0.038
Site #1 Test	<250 µm	3,418	0.0136	0.046	0.387	0.100	0.039
Site #1 Test	<38 µm	5,328	0.0121	0.064	0.445	0.100	0.045
Site #1 Test	<38 µm	5,328	0.0110	0.059	0.426	0.100	0.043
Site #1 Test	<38 µm	5,328	0.0131	0.070	0.470	0.100	0.047
Site #2 Test	<2 mm	20,762	0.0113	0.235	0.948	0.100	0.095
Site #2 Test	<2 mm	20,762	0.0126	0.262	1.154	0.100	0.115
Site #2 Test	<2 mm	20,762	0.0107	0.222	1.109	0.100	0.111
Site #2 Test	<250 µm	32,175	0.0118	0.380	1.533	0.100	0.153
Site #2 Test	<250 µm	32,175	0.0110	0.354	1.278	0.100	0.128
Site #2 Test	<250 µm	32,175	0.0106	0.341	1.253	0.100	0.125
Site #2 Test	<38 µm	56,732	0.0103	0.584	2.959	0.100	0.296
Site #2 Test	<38 µm	56,732	0.0107	0.607	2.706	0.100	0.271
Site #2 Test	<38 µm	56,732	0.0111	0.630	2.836	0.100	0.284
Site #3	<38 µm	11,537	0.0162	0.187	0.836	0.100	0.084
Site #4	<38 µm	14,501	0.0132	0.192	1.550	0.100	0.155

Notes:

a. Based on XRF measurements.

b. Concentrations have been corrected for filter contamination.

Table 5. Bioaccessibility Data Summary - Royal Roads

Soil ID	Grain Size (µm)	Barium Conc. in Soil [a] (mg/kg)	Mass of Soil Tested (g)	Mass of Barium in Soil Extracted [a] (mg)	Barium Conc. in Extract [b] (mg/L)	Volume of Extract (L)	Mass of Barium in Extract (mg)
Solid to Fluid Ratio = 1:100							
Site #2 Test	<45	56,732	0.9925	56.30667003	9.7	0.100	0.97
Site #2 Test	<45	56,732	1.0032	56.91370415	9.79	0.100	0.979
Site #2 Test	<45	56,732	1.0287	58.36037427	10.1	0.100	1.01
Site #3 Test	<45	11,537	1.005	11.594685	11.9	0.100	1.19
Site #3 Test	<45	11,537	1.0012	11.5508444	11.9	0.100	1.19
Site #3 Test	<45	11,537	1.0074	11.6223738	11.5	0.100	1.15
Solid to Fluid Ratio = 1:1,000							
Site #2 Test	<45	56,732	0.1035	5.871778688	11.3	0.100	1.13
Site #2 Test	<45	56,732	0.1163	6.597950352	11.4	0.100	1.14
Site #2 Test	<45	56,732	0.1001	5.67888934	11.4	0.100	1.14
Site #3 Test	<45	11,537	0.1022	1.1790814	6.71	0.100	0.671
Site #3 Test	<45	11,537	0.1003	1.1571611	6.42	0.100	0.642
Site #3 Test	<45	11,537	0.1009	1.1640833	6.77	0.100	0.677
Site #3 Test	<250	11,537	0.12	1.38444	5.51	0.100	0.551
Site #3 Test	<250	11,537	0.1009	1.1640833	4.92	0.100	0.492
Site #3 Test	<250	11,537	0.1013	1.1686981	4.76	0.100	0.476
Site #3 Test	<125	11,537	0.1013	1.1686981	5.29	0.100	0.529
Site #3 Test	<125	11,537	0.1095	1.2633015	5.62	0.100	0.562
Site #3 Test	<125	11,537	0.1	1.1537	5.65	0.100	0.565
Site #3 Test	<63	11,537	0.1036	1.1952332	6.15	0.100	0.615
Site #3 Test	<63	11,537	0.1057	1.2194609	6.23	0.100	0.623
Site #3 Test	<63	11,537	0.1087	1.2540719	6.42	0.100	0.642
Site #3 Test	<45	11,537	0.108	1.245996	6.87	0.100	0.687
Site #3 Test	<45	11,537	0.1003	1.1571611	6.7	0.100	0.67
Site #3 Test	<45	11,537	0.1008	1.1629296	6.67	0.100	0.667
Site #4 Test	<250	14,501	0.1007	1.4602507	4.11	0.100	0.411
Site #4 Test	<250	14,501	0.1107	1.6052607	4.23	0.100	0.423
Site #4 Test	<250	14,501	0.1135	1.6458635	4.34	0.100	0.434
Site #4 Test	<125	14,501	0.1045	1.5153545	4.94	0.100	0.494
Site #4 Test	<125	14,501	0.1274	1.8474274	5.37	0.100	0.537
Site #4 Test	<125	14,501	0.1103	1.5994603	5.18	0.100	0.518
Site #4 Test	<63	14,501	0.1149	1.6661649	5.59	0.100	0.559
Site #4 Test	<63	14,501	0.1222	1.7720222	5.79	0.100	0.579
Site #4 Test	<63	14,501	0.1165	1.6893665	5.52	0.100	0.552
Site #4 Test	<45	14,501	0.1209	1.7531709	5.87	0.100	0.587
Site #4 Test	<45	14,501	0.106	1.537106	5.66	0.100	0.566
Site #4 Test	<45	14,501	0.1205	1.7473705	6.07	0.100	0.607

Table 5. Bioaccessibility Data Summary - Royal Roads

Soil ID	Grain Size (µm)	Barium Conc. in Soil[a] (mg/kg)	Mass of Soil Tested (g)	Mass of Barium in Soil Extracted[a] (mg)	Barium Conc. in Extract[b] (mg/L)	Volume of Extract (L)	Mass of Barium in Extract (mg)
Solid to Fluid Ratio = 1:10,000							
Site #2 Test	<45	56,732	0.0412	2.337365043	3.27	0.100	0.327
Site #2 Test	<45	56,732	0.0422	2.394097204	3.27	0.100	0.327
Site #2 Test	<45	56,732	0.0442	2.507561527	3.24	0.100	0.324
Site #3 Test	<45	11,537	0.0425	0.4903225	0.79	0.100	0.079
Site #3 Test	<45	11,537	0.0412	0.4753244	0.94	0.100	0.094
Site #3 Test	<45	11,537	0.0431	0.4972447	0.52	0.100	0.052

Notes:

a. XRF analyses not included in Royal Roads scope of work - XRF analyses from Exponent data.

b. Royal Roads data for Site 5 not included as no XRF analytical data were available

Figure 1. Barium in Extract as a Function of Barium in Soil

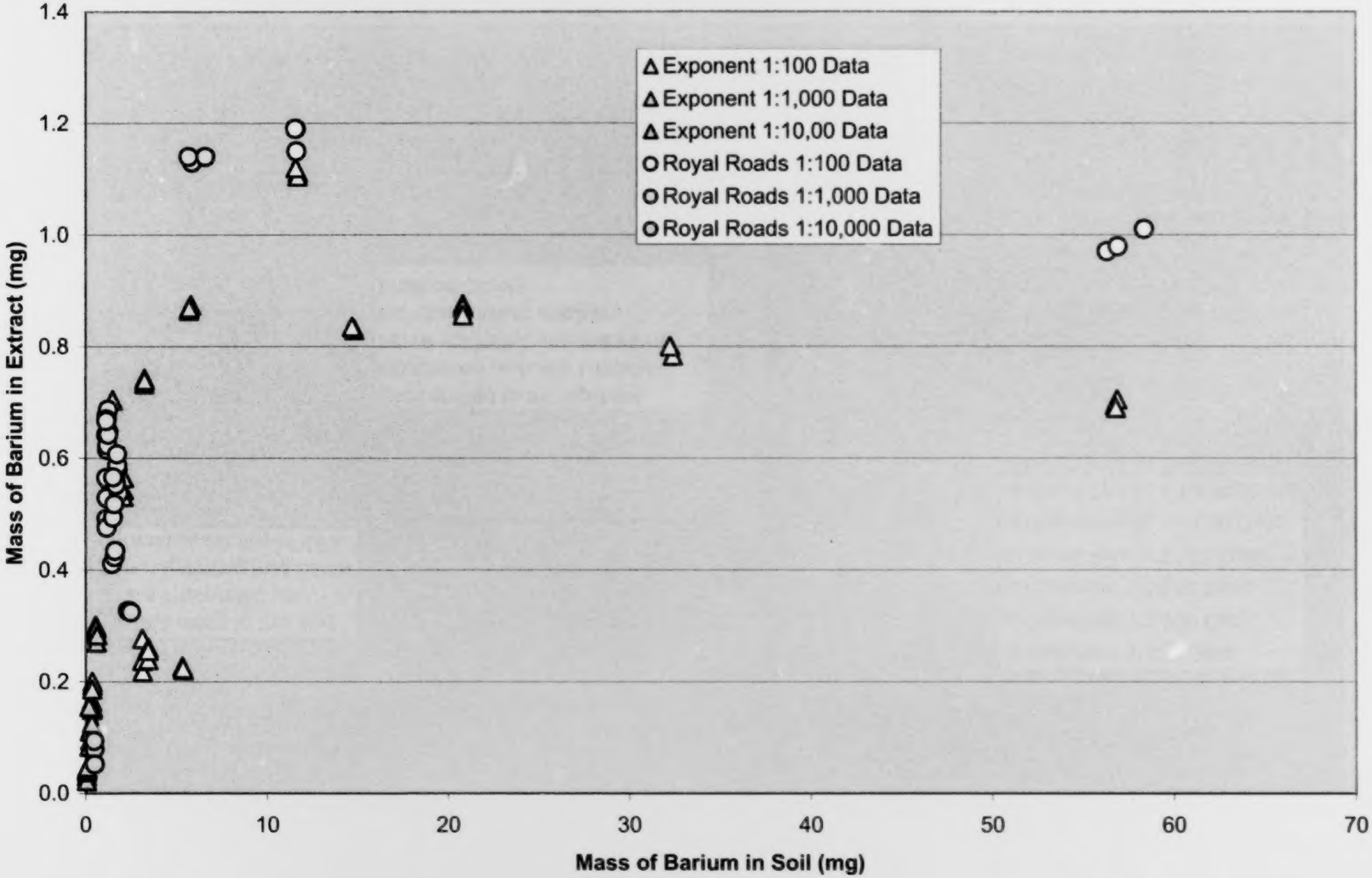
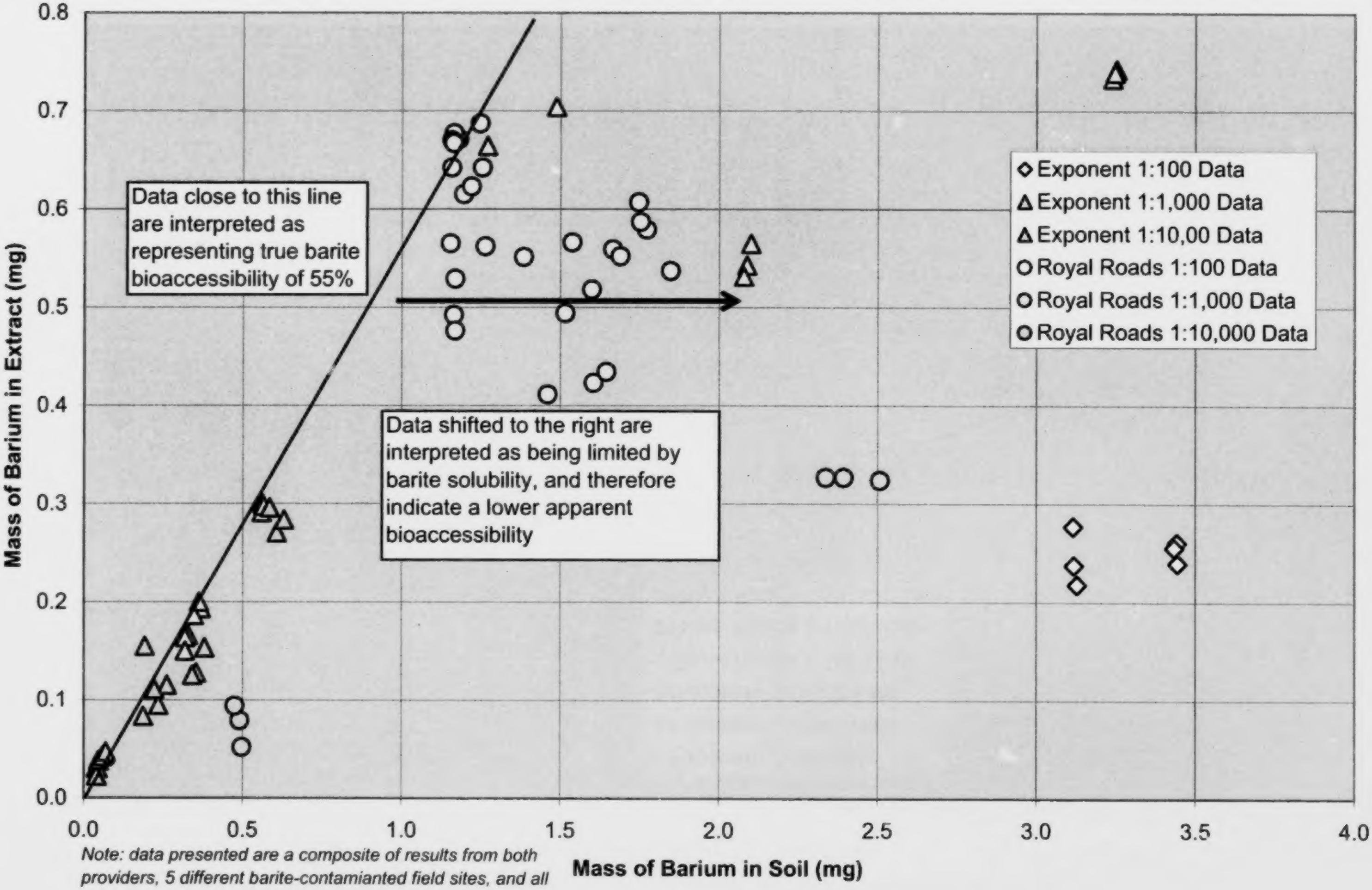


Figure 2. Barium Extraction Data Interpreted as Bioaccessibility



Data close to this line are interpreted as representing true barite bioaccessibility of 55%

Data shifted to the right are interpreted as being limited by barite solubility, and therefore indicate a lower apparent bioaccessibility

Note: data presented are a composite of results from both providers, 5 different barite-contamianted field sites, and all available grain sizes and repetitions

APPENDIX A

ROYAL ROADS BIOACCESSIBILITY STUDY

BIOACCESSIBILITY TESTING OF BARITE IN SUPPORT OF REFINING SOIL QUALITY GUIDELINES FOR BARITE

ROYAL ROADS
UNIVERSITY

Report Prepared for:

Canadian Association of Petroleum Producers
Suite 2100, 350 7th Avenue SW
Calgary, Alberta T2P 3N9

By:

School of Environment & Sustainability
Royal Roads University
2005 Sooke Road
Victoria, British Columbia, Canada
V9B 5Y2

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1. INTRODUCTION

Barite (barium sulfate) is frequently used as a weighting agent in oilfield drilling muds due to its high specific gravity. This is usually accomplished by pumping a mixture of crushed barite, water and other materials into the test hole. Some of the barite remains on site after drilling, resulting in residual soil barium concentrations that can exceed the Canadian Council of Ministers of the Environment (CCME) soil quality guidelines for barium (CCME, 1999). The current CCME soil quality guidelines were developed based on highly water soluble and therefore, highly bioavailable forms of barium, such as barium chloride (solubility of 0.37 g/mL), whereas barite is virtually insoluble (solubility of 2.2µg/mL). There are currently no published suitable bioavailability studies for barite.

In vivo studies have traditionally been used to estimate metal bioavailability (Ruby et al, 1999; Kelly et al, 2002). Due to the expense and time constraints involved in these in vivo studies, in vitro digestion models using Physiologically Based Extraction Tests (PBET) have been used as surrogates for bioavailability. PBET models typically measure bioaccessibility, which is the fraction of the contaminant that becomes dissolved in the digestive tract fluids and is assumed to be available for absorption (Ruby et al, 1999). It is determined from the in vitro assay as the ratio of the mass of contaminant dissolved into a volume of simulated gastric fluid to the original mass of contaminant in the soil sample.

A literature review on bioavailability indicates in vitro bioaccessibility assays have been validated for lead, arsenic and cadmium using in vivo animal models (Ruby et al., 1999; Batelle, 2000). In vitro bioaccessibility tests have also been conducted for chromium, mercury and nickel but are yet to be validated using in vivo models. There are no literature references for the determination of the bioaccessibility of barium in barite. This is presumably due to the low aqueous solubility of barite. Accidental ingestion of barium salts can lead to their solubilization in water and stomach acids leading to cases of poisoning and in some instances death (Hans and Sigel, 1998). Toxicity depends on the solubulization of the salt and hence, its chemical speciation. Acid-soluble barium compounds, such as barium chloride and barium carbonate, are absorbed through the gastrointestinal tract, although the amount of barium absorbed is highly variable. Barium sulfate is extremely insoluble and very little, if any, ingested barium sulfate is absorbed. (ATSDR, 2006). Thus, the extent to which small amounts of barite would become solubilized in the acids and enzymes in a mammalian stomach (i.e., what fraction of the barite is bioaccessible) is unknown. The main objective of this report is to determine a conservative estimate of the bioaccessibility of barite, and to use this estimate to refine the barite soil quality guidelines for human soil ingestion and for livestock/wildlife soil ingestion.

There are various in vitro digestion models for bioaccessibility; Oomen et al (2002) conducted a multi-laboratory comparison and evaluation of five in vitro digestion models. A wide range of of bioaccessibility values were found for the three soil samples evaluated: As (6 – 95%, 1 – 19%, 10 – 59%); Cd (7 – 92%, 5 – 92%, 6 – 9%); and Pb (4 – 91%, 1 – 56%, 3 – 90%). This was attributed to differences in experimental design including applied gastric pH, different liquid -to- solid ratio and different filtration procedures. Recent reviews by Bright et al., (2006) and Richardson et al. (2006) also identified the following concerns:

- Standard in vitro methods for measuring bioaccessibility do not normally consider the particle size likely to be ingested, but simply test <250μm soil samples from the site in question;
- Standard in vitro methods for measuring bioaccessibility do not reflect the typical conditions of the gastric environment which may range from gastric fluid (in mL) to soil (in g) ratios of about 12,500:1 to 2,500:1; and,
- In vitro bioaccessibility methods may, in some cases, measure solubility saturation rather than true solubility

This report presents the results of investigations on the bioaccessibility of barite under various conditions that included particle size and extraction liquid-to-soil ratio.

2. METHODOLOGY

2.1 DIGESTION MODEL

Experiments were conducted to assess two digestion models for barite bioaccessibility: these included the Simplified Bioaccessibility Extraction Test (SBET); and a Sequential Stomach and Small Intestinal Phase (Gastrointestinal – GI) model.

The SBET simulates the mobilization of contaminants in the acid conditions of the stomach and is based on the work of the Solubility/Bioavailability Research Consortium (Exponent & Batelle, 2000). The protocol involves a 1-h extraction (mixing by end-over-end rotation at 37°C) of 1 g of substrate in 100 mL of HCl and glycine buffer at pH 1.5. For this study, 1.00 ±0.05 g of 98% barite (Sigma-Aldrich) or National Institute of Standards and Technology (NIST) Standard Reference Material (NIST 2711) was weighed and placed into a 125 mL polyethylene extraction bottle and 100 ± 0.5 mL of extraction solution was added. The extraction solution consisted of 30 g/L glycine (Calbiochem) adjusted to a pH of 1.5 with concentrated HCl (E M Science, OmniTrace). Extraction was conducted in triplicates for barite and NIST 2711. The bottles were placed into the extractor in batches of eight and rotated end-over-end in a 37°C water bath for 1h. After the extraction was completed, the bottles were removed. Each extract was drawn directly into a disposable 20 mL plastic syringe with a luer slip (National Scientific). A 0.45µm cellulose acetate filter (25 mm diameter, Cole Palmer) was attached to the syringe and the extract was filtered into a clean 20 mL polyethylene scintillation vial. The filtered extract was stored at 4°C and subsequently analyzed for metals by ICP-OES. The results obtained are given in Tables A.1 and A.2 in the Appendices; batch summaries that include initial and final pH of extracts are included in Appendix B, Table B.1.

The Sequential Stomach and Small Intestinal Phase Extraction (Gastrointestinal - GI) model is designed to determine the fraction of the metal that is solubilized and therefore available for absorption in both the stomach and small intestine (gastrointestinal tract) and is based on methods described in Exponent & Batelle (2000). The barite or NIST 2711 sample (1.00 ± 0.05 g) was placed into a 150 mL extraction vessel containing 100 mL of the stomach extraction solution. The stomach solution consisted of a mixture pepsin, citrate, malate, lactic acid, acetic acid and HCl at a pH of 2 as described in

Exponent & Batelle (2000). The mixture was incubated in a water bath at 37°C for 10 min. The pH of each solution was determined after 5 min and adjusted to 2 where necessary. The solution was rotated end-over-end for 1 hr in the water bath. After which a 10 mL aliquot was collected and filtered through a 0.45 µm cellulose acetate filter. The pH of the remaining solution was adjusted to 7.0 ± 0.2 with saturated sodium bicarbonate solution followed by addition of bile salts and pancreatin to mimic intestinal conditions. The solution was rotated end-over-end for another 3 hrs after which a 20 mL aliquot was collected and filtered through 0.45µm cellulose acetate filter. The filtrates from both the stomach phase and the intestinal phase were analyzed for barium by ICP-OES. Data is given in Table A.1 and A.2; laboratory batch summary can be found in Appendix B, Table B.3.

2.2 EFFECT OF PH, PARTICLE SIZE AND DILUTION RATIO ON BARITE BIOACCESSIBILITY

Based on the results of the initial extraction tests (Appendix A, Table A.1) which suggested comparable results between the simplified bioaccessibility extraction test and sequential stomach and small intestinal phase extraction model, the simplified extraction test was selected for all subsequent experiments.

The effect of pH on barite bioaccessibility was investigated by conducting extractions in 30 g/L glycine adjusted with concentrated HCl to a pH of 1.1, 1.5, 2.0, 3.0 or 4.0 (see Tables B1 and B2 for batch summaries). This was followed by studies on particle size effects during which pure barite was mechanically sieved into four size fractions as follows: <250 µm, <125 µm, <63 µm and <45 µm. Barium bioaccessibility for each fraction was determined in triplicate using an extraction solution-to-solid ratio of 1000:1. The batch summaries for these extractions are given in Tables B.4 and B5. In order to determine whether the bioaccessible fraction of barite changed with extraction solution-to-solid ratio, extractions were conducted using the following ratios - 100:1, 500:1, 1000:1, 2000:1, 5000:1 and 10000:1 (see Tables B.6 and B.7) for the laboratory batch summaries.

For additional investigation into dilution effects, 1 g of barite was added to 50 g of Organization for Economic Co-operation and Development (OECD) artificial soil. The OECD soil was prepared according to OECD Guideline 207 (OECD, 1984) and was composed (by weight) of 70% sand (Lane Mountain silica sand LM#70, Target Products Ltd.), 20% kaolinite clay (Lloyd-El Ceramics and Crafts Ltd.) and 10% peat (Marigold Nurseries Ltd.). Extractions were conducted in triplicate using 1 g of the soil and 100 mL of the extraction solution at a pH of 1.5 (see Table B.5 for the bath summary).

Total metals including barium in soil samples were determined by acid digestion using procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846 Method 3051, published by the United States Environmental Protection Agency (EPA). Instrumental analysis was by inductively coupled plasma - optical emission spectrophotometry (ICP-OES) (EPA Method 6010B). Extracts were analyzed directly by ICP-OES.

2.3 BARIUM BIOACCESSIBILITY IN BARITE CONTAMINATED FIELD SOIL SAMPLES

This phase of the investigation involved measuring the bioaccessibility of barium in barite contaminated field soils samples using the SBET protocol. The effects of varying soil particle size and extraction solution-to-soil ratios were also studied. Since natural soils may contain different species of barium compounds that may be much more bioaccessible than barite, background soil samples were also collected from the sites and used as control soils.

Soil samples from four oilfield sites with differing soil types and known to be contaminated with drilling mud containing barite were collected and shipped to the laboratory. Background samples were also obtained from three of the sites. Each soil sample was homogenized in a stainless steel bowl and dried overnight in an oven at <40 °C prior to analysis (Appendix B, Table B.8).

Each dried soil sample was sieved using USA Standard Testing Sieve ASTM E11 series and a Meinzer II Sieve Shaker into the following size fractions: <250 µm, <125 µm, <63 µm and <45 µm (Appendix B, Table B.9). Total barium concentrations in each of the four soil particle size fractions were determined in triplicate as described above for Site 3 and 4. Barium bioaccessibility for Site 3 and 4 was measured in triplicate for each fraction using the SBET protocol. Extraction was conducted with 0.100 ± 0.05 g of soil and 100 ± 0.5 mL aliquot of the extraction solution at a pH of 1.5 (Tables B.9 to 12). The filtered extract was stored at 4°C and subsequently analyzed for barium as above.

Sub-samples of the sieved soils from Site 4 were also analyzed for total metals, pH and total organic carbon (TOC). Total metals were analyzed by ICP-OES as described above; pH was determined by the slurry method by mixing the dried sample with distilled water at a ratio of 1:2. The pH of the solution was then measured using a standard pH meter (WTW MultiLine P3 equipped with a SenTix 41-3 probe). Total organic carbon was measured in accordance with USEPA Method 9060A using a non dispersive infra red detector.

Dried soil samples from study Sites 2, 3, 4 and 5 were sieved though <45 µm sieves and extractions were conducted using the extraction solution-to-soil ratios of 100:1, 1000:1 and 10000:1. These three ratios were achieved by using 100 mL of extraction solution and 1 g soil, 100 ml extraction solution and 0.1 g soil and 500 ml extraction solution and 0.05 g of soil, respectively. The extractions were conducted in triplicate at each dilution ratio. The batch summaries for these extractions are given in Tables Total barium in soils and extracts were determined by ICP-OES as described above.

Finally, samples from the four sites were analyzed for barium using the Alberta extractable barium method (Alberta Environment, 2004). The sub-sample of the air died sample was sieved to <2 mm. A 10 g portion was weighed into a 125 mL polyethylene bottle and 100 mL of 0.1 M calcium chloride was added. The solution was rotated end-over-end for 2 h. The resulting solution was filtered under gravity. The extract was analyzed for barium by ICP-OES and the result was converted to mg/kg dry soil.

2.4 STATISTICAL ANALYSES

Statistical analyses were conducted using Mintab® 14. Linear regression was used to determine correlation between parameters while analysis of variance, followed by Tukey's pairwise comparisons (ANOVA), was used to determine the agreement between multiple tests.

3. RESULTS AND DISCUSSIONS

3.1 DIGESTION MODEL

A summary of the barium bioaccessibility obtained for pure barite and NIST 2711 using the two digestion models is given in Table 1 below. Detailed results are provided in Appendix A, Tables A.1 and A.2, respectively.

Table 1: Barium Bioaccessibility (± Standard Deviation, *n = 3*) for Barite and NIST 2711 using the SBET and GI Digestion Models with Solution-to-Solid Ratio of 100:1

Method	Barium Bioaccessibility (%)	
	Barite	NIST 2711
SBET	0.13 ± 0.004	19.6 ± 0.9
GI – Stomach phase	0.048 ± 0.001	17.0 ± 0.9
GI – Intestinal phase	0.067 ± 0.007	9.9 ± 1.1

The mean barium bioaccessibility for pure barite of 0.13 % using the SBET protocol was higher than the value obtained for the GI model which was 0.048% for the stomach phase and 0.067% for the intestinal phase. Barium bioaccessibility for NIST 2711 in the GI stomach phase was however, comparable to that obtained using the SBET model. Since comparable results were obtained for NIST 2711, and the SBET procedure was conservative (higher bioaccessibility than the sequential method) for barite, the SBET model was selected for further studies including the effect of pH, particle size and extraction solution-to-solid ratio.

3.2 EFFECT OF EXTRACTION SOLUTION PH ON BARITE BIOACCESSIBILITY

The bioaccessibility of barium for pure barite, using the SBET and extraction solution-to-solid ratio of 100:1, decreased with increasing pH as illustrated in Figure 1. Detailed results are given in the Appendices in Table A.3. Stomach pH is known to range from 1 under fasting conditions to 4 after a meal due to the buffering capacity of proteins. The data obtained suggested that the role of food in the absorption process may need to be considered in the use of the barite bioaccessibility data.

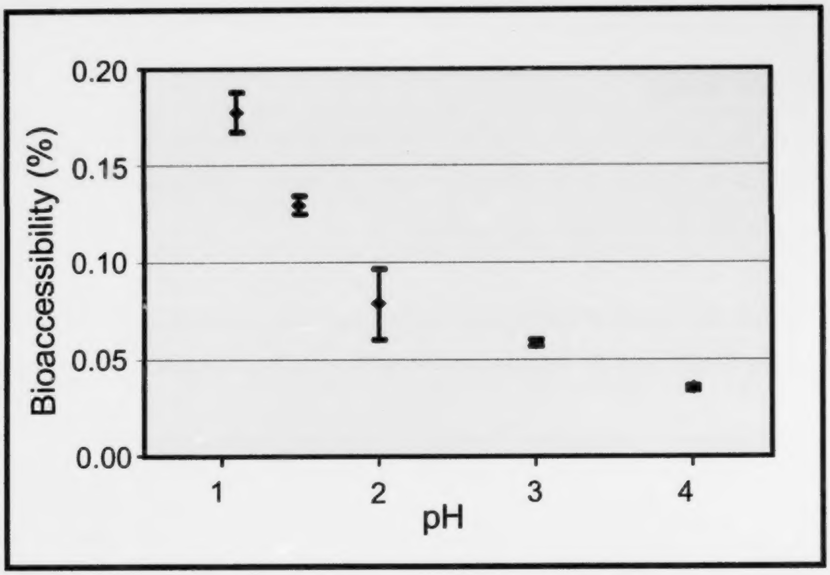

Figure 1: Effect of Extraction Solution pH on Barium Bioaccessibility for Pure Barite using SBET with Solution-to-Solid Ratio of 100:1

3.3 EFFECT OF EXTRACTION SOLUTION-TO-SOLID RATIO ON BARITE BIOACCESSIBILITY

The bioaccessibility of barite increased with increasing extraction solution-to-solid ratio as illustrated in Figure 2. Detailed results are provided in Appendices in Table A.4. There was a linear relationship ($y = 0.0013x + 0.44$, $R^2 = 0.9404$) and the data indicated there was a hundred-fold increase in bioaccessibility when the ratio was increased from the usual 100:1 to 10,000:1. This may be attributed to saturation effects at the lower dilution ratios.

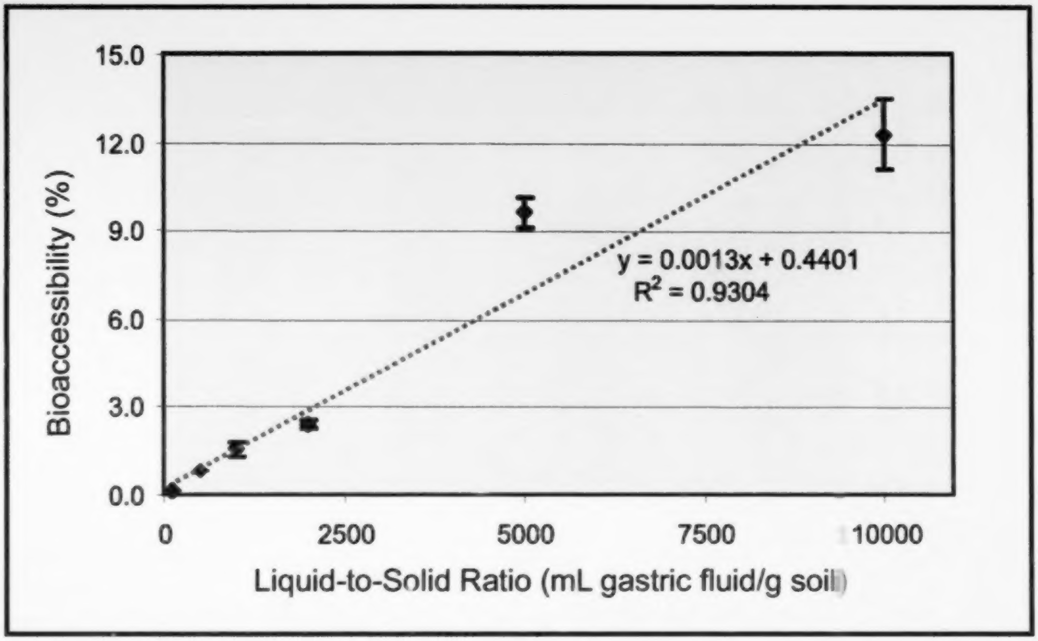

Figure 2: Effect of Liquid-to-Solid Ratio on Barium Bioaccessibility for Pure Barite

3.4 EFFECT OF PARTICLE SIZE ON BARITE BIOACCESSIBILITY

Using a dilution ratio of 1000:1, extractions were conducted for pure barite sieved to four particle size fractions as follows: <250 µm, <125 µm, <63 µm and <45 µm. Barium bioaccessibility in the four different size fractions were comparable, as illustrated in Figure 3. Complete results are given in Table A.5. An ANOVA followed by Tukey's pairwise comparison (see Appendix C.1 for detailed results) yielded F= 3.04 and p = 0.093 indicating there was no statistically significant difference between barium bioaccessibility for the different barite particle size fractions.

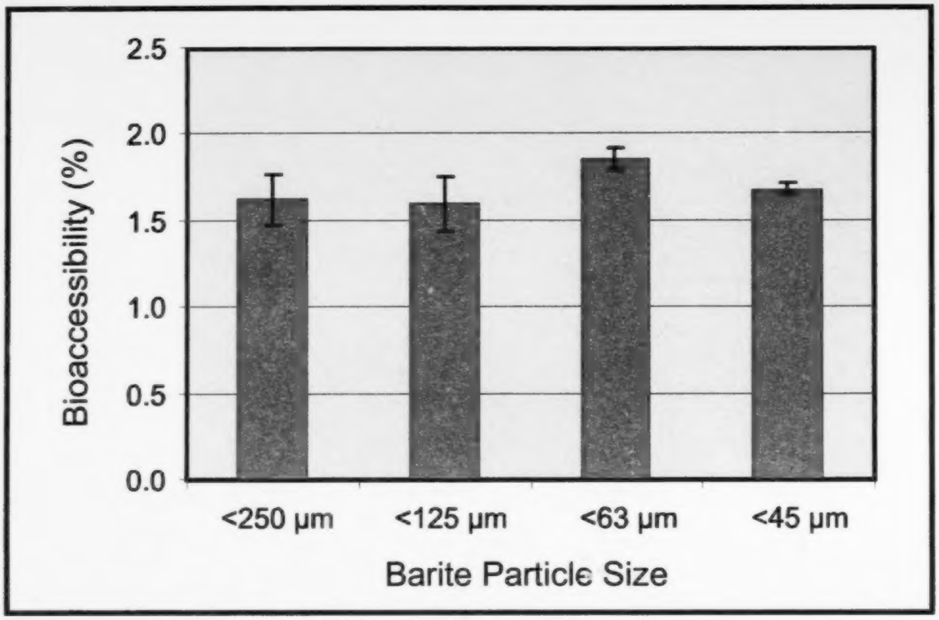

Figure 3: Variation of Barium Bioaccessibility as a Function of Barite Particle Size as Determined by SBET with Solution-to-Solid Ratio of 1000:1

3.5 MATRIX EFFECTS

For additional investigation into matrix effects, 1 g of barite was added to 50 g of OECD artificial soil. Barium bioaccessibility for the mixture was then determined using 1 g of the soil and 100 mL extraction fluid. The mean barium bioaccessibility was determined to be 12.2 ± 0.2 % (see Table A.6 for complete results) compared to 0.13 % for the pure barite (without the OECD soil) at the 100:1 dilution ratio. The increase in bioaccessibility may be attributed to the dilution effect of the matrix. Assuming barite was distributed evenly in the OECD soil, the 1 g sample size used for extraction contained 0.02 g of pure barite resulting in a solution-to-solid barite ratio of 100 mL:0.02 g or 5000:1. The bioaccessibility of pure barite at the 5000:1 dilution ratio was 9.6 ± 0.5 % while at the 10,000:1 it was 12.2 ± 1.2% (see Figure 2).

3.6 BARIUM BIOACCESSIBILITY IN BARITE CONTAMINATED FIELD SOIL SAMPLES

3.6.1 Particle Size Effects

This investigation began with the assessment of barium bioaccessibility in samples from Site 3 and 4 using the SBET protocol at a pH of 1.5, but an extraction solution-to-solid ratio of 1000:1 to allow for a better detection. The mean bioaccessibility and standard deviation obtained for four soil particle size fractions are given in Table 2; the complete results are available in Table A.7 and Table A.8 in the Appendices. Bioaccessibility generally increased with decreasing soil particle size, with the <45µm fraction having the highest values. The difference in bioaccessibility was statistically significant for each site based on the ANOVA as presented in Appendix B.2 and B.3 with p = 0.009 for Site 3 and p =0.001 for Site 4.

Table 2: Barium Bioaccessibility for Barite Contaminated Oilfield Soil Samples using the SBET with Solution-to-Solid Ratio of 1000:1

Soil Particle Size	Barium Bioaccessibility (%)	
	Site 3	Site 4
Control, <250 µm	7.96 ± 0.04	9.19 ± 0.33
<250 µm	30.7 ± 5.0	32.7 ± 3.8
<125 µm	33.8 ± 3.4	50.7 ± 3.0
<63 µm	39.8 ± 3.5	47.9 ± 3.1
<45 µm	45.3 ± 2.2	57.1 ± 6.5

Total barium concentration in the different soil particle size fractions for the two test sites are shown in Figure 4. There was no statistically significant difference between the total barium concentrations in the different soil particle sizes for Site 3 (see Appendix B.4 for the ANOVA) while the concentrations decreased slightly with decreasing soil particle size for Site 4 (see Appendix B.5 for the ANOVA). Based on these observations, the increase in bioaccessibility with decreasing soil particle size may be attributed to the increased amount of barium extracted from the smaller particles. Smaller particles have a greater surface area to volume ratio and thus interact with the solution more effectively, leading to an increase in bioaccessibility. This is particularly true for less soluble compounds, which dissolve by surface-reaction-controlled kinetics (Ruby et al, 1999).

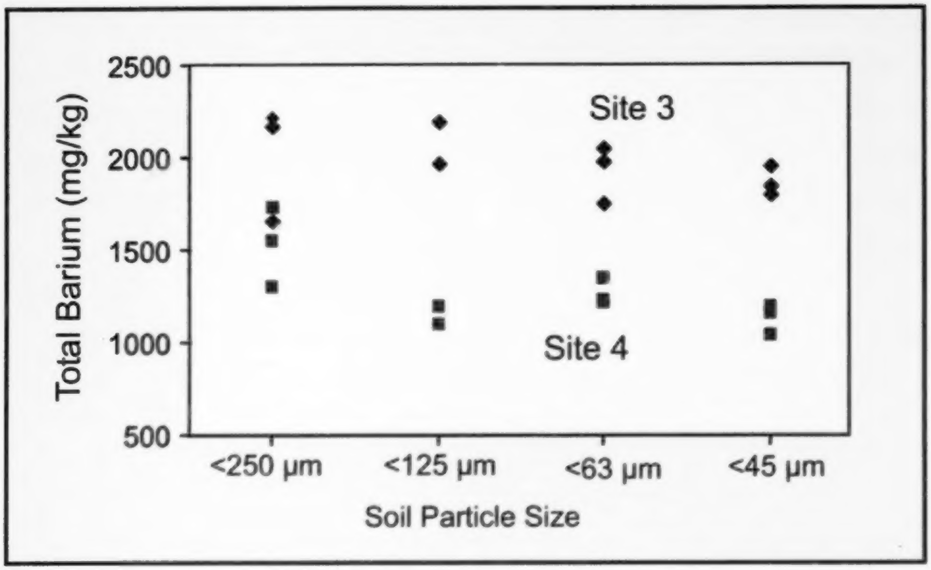

Figure 4: Total barium in soil as a function of soil particle size fraction

Apart from the form of the metal and soil particle size, it has been suggested that bioavailability is dependent on various physical and chemical characteristics of the soil including oxidation-reduction conditions (especially in sediments), total organic carbon (TOC), pH and cation exchange capacity (CEC) (Kelly et al, 2002; NRC; 2002). An investigation into the effect of pH, TOC and total metal distribution was conducted for one site (Site 4 was arbitrarily chosen for the study). Total metals concentrations, pH and TOC of the four particle size fractions are given in Appendix A, Table A.9, while data for the control soil is presented in Table A.10. Apart from barium, total metal concentrations were generally below 100 mg/kg with the concentrations of copper and zinc showing a slight increase with decreasing particle size (Figure 5).

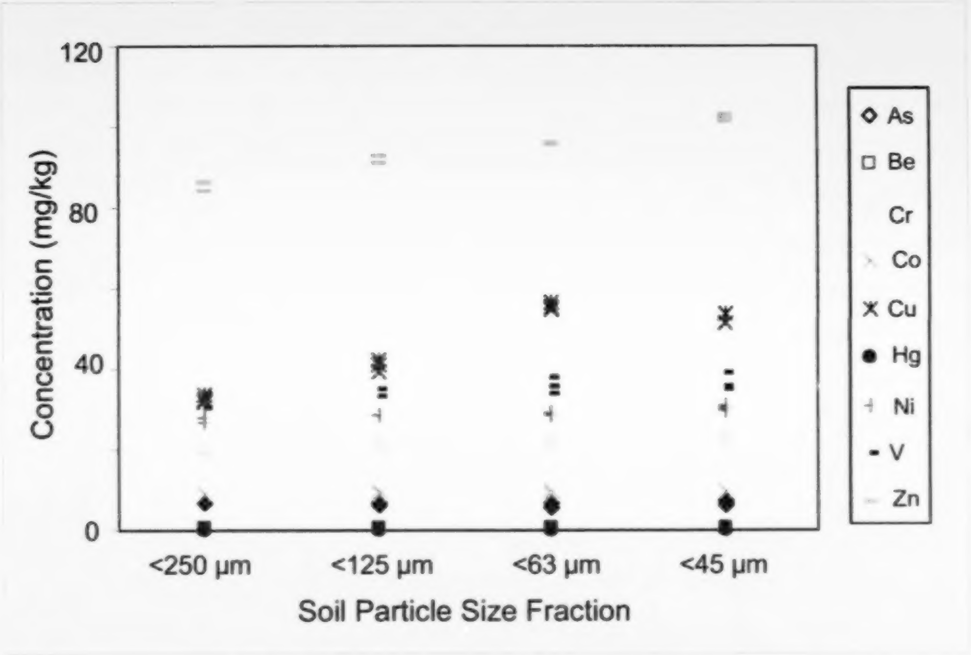

Figure 5: Total metals in soil as a function of soil particle size fraction

There was an increase in barium bioaccessibility with increasing soil TOC as depicted in Figure 6. A linear correlation yielded a Pearson correlation coefficient of 0.866, p<0.001 suggesting a strong relationship.

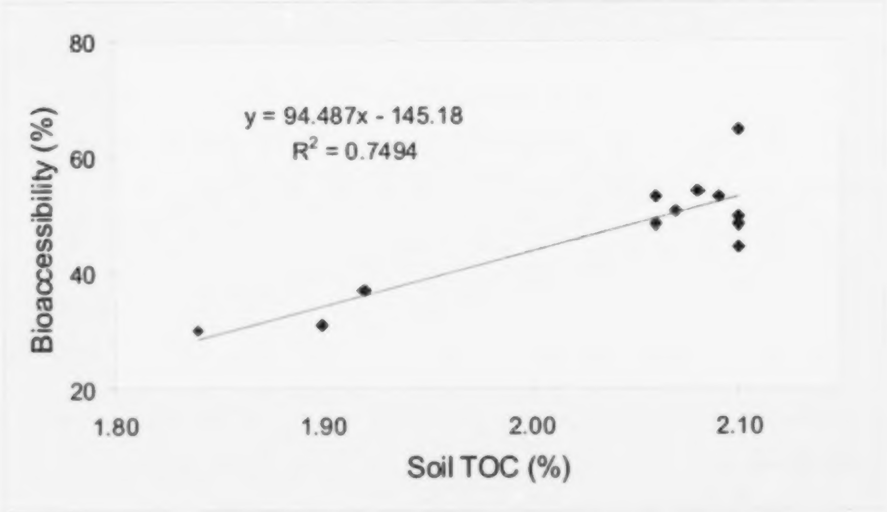

Figure 6: Barium Bioaccessibility for Site 4 Determined by SBET with Solution-to-Solid Ratio of 1000:1 as a function of soil TOC

In general metals are less available at higher TOC concentrations due to the ability of metals to form complexes with organic materials that are not easily dissociated. For the Site 4 soil sample, barium bioaccessibility increased with increasing TOC, indicating that organic matter binding may not be influential in the bioaccessibility. The increase in bioaccessibility with increasing TOC may be due to particle size interactions in that the smaller particle size fractions had higher TOC concentrations as illustrated in Figure 7. A linear regression yielded a Pearson correlation coefficient of -0.880, p<0.000 indicating a strong relationship.

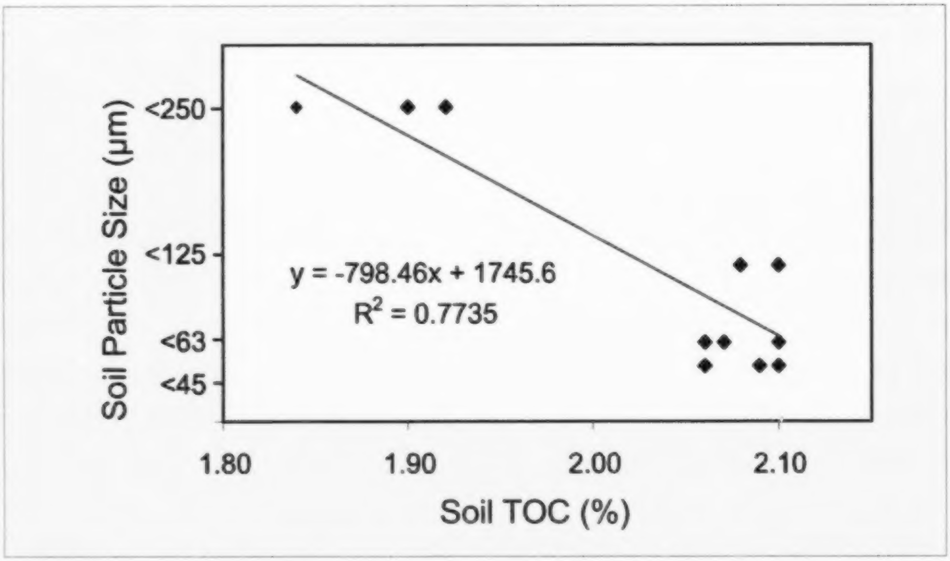

Figure 7: Soil particle size fraction as a function of soil total organic carbon (TOC)

There was no discernible relationship between soil pH and bioaccessibility (Figure 8) with Pearson correlation coefficient of 0.031, p= 0.924.

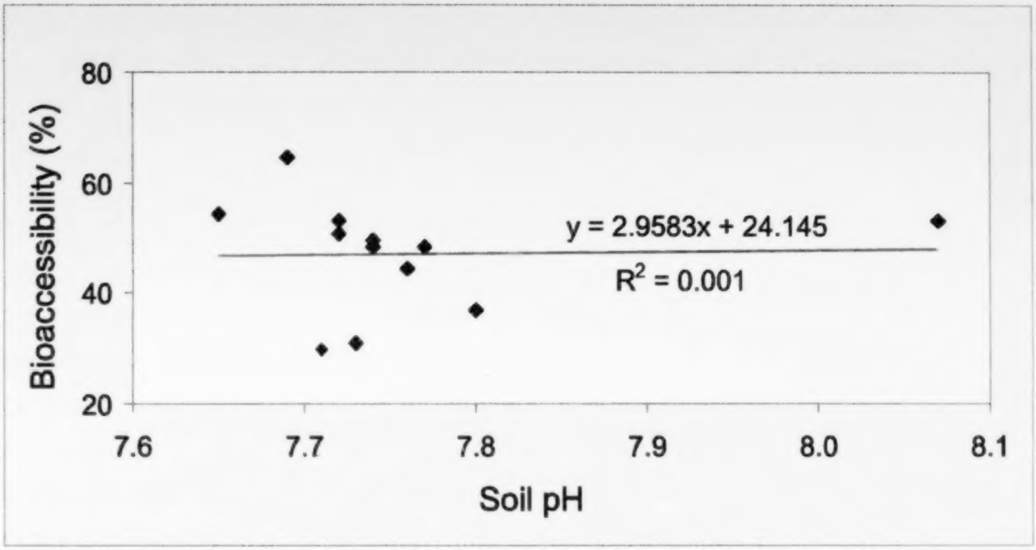

Figure 8: Barium bioaccessibility as a function of soil pH

3.6.2: Effect of Extraction Solution-to-Soil Ratio on Bioaccessibility

Investigation on extraction solution dilution effects were conducted for Sites 2, 3, and 5 using the <45 µm soil particle size fraction. The mean barium bioaccessibility for the sites, as presented in Table 3, indicated that bioaccessibility increased with increasing dilution ratio as found for the pure barite sample. Detailed results are given in Tables A.13 to A15.

Table 3: Barium Bioaccessibility for Barite Contaminated Oilfield Soil Samples using the SBET and Different Solution-to-Solid Ratios

Extraction Solution-to-Soil Ratio	Barium Bioaccessibility (%)		
	Site 2*	Site 3	Site 5
100:1	1.72 ± 0.01	8.09 ± 0.18	6.69 ± 0.26
1000:1	18.9 ± 1.4	45.3 ± 1.1	25.3 ± 0.45
10000:1	55.1 ± 2.1	49.4 ± 14	33.0 ± 2.3

* Bioaccessibility was calculated using total barium concentration determined by X-ray fluorescence analysis

Barium bioaccessibility for Site 2 was calculated using total barium concentration as determined by X-ray fluorescence analysis rather than ICP-OES. The values calculated for Site 2 using the ICP-OES data ranged from 11.4% to 371%. The values of over 100% obtained for Site 2 was attributed to the inability of the digestion method employed to recover all the barium into acid solution for subsequent ICP-OES analysis. Sub-samples from Sites 2, 3 and 4 were subsequently analyzed for total barium by X-ray fluorescence by Exponent. A comparison of the concentrations as determined by XRF and ICP-OES is given in Table A.12. The results obtained by the two techniques were in the same order of magnitude for Sites 3 and 4, however for Site 2 barium concentration showed a difference of over 60%. Bioaccessibility for Site was therefore re-calculated using the XRF data as given in Table A.13.

3.7 Extractable Barium

The concentrations of extractable barium in the four samples using the Alberta extractable barium method (Alberta Environment, 2004) are given in Table 4.

Table 4: Concentration of Extractable Barium in Barite Contaminated Oilfield Soil Samples

Sample ID	Extractable Barium (mg/kg)			
	Site 2	**Site 3**	**Site 4**	**Site 5**
Extractable Barium	22.1	18.8	12.5	12.3

4. SUMMARY OF FINDINGS

Given the similarity of initial tests comparing bioaccessibility of barite using the Simplified Bioaccessibility Extraction Test (SBET) and the Sequential Stomach and Small Intestinal Phase (Gastrointestinal – GI) method, the SBET procedure was employed exclusively for subsequent testing procedures for reasons of reduced labor and time requirements.

Investigations into the testing parameters of the SBET model using samples of pure barite indicated some variability in results based on the extraction conditions used. An increase in bioaccessibility was identified for increased extraction solution-to-ratio, while an increase in pH was shown to result in decreased bioaccessibility. There was no significant difference in bioaccessibility for barite samples sieved to different particle sizes. The addition of an OECD soil matrix to the barite sample resulted in an increased bioaccessibility, most likely attributed to an overall dilution effect.

The SBET method was further applied to soil samples collected from known sites of barite contamination to examine the interactions of the physical characteristics of soil and resulting bioaccessibility. Unlike the pure barite samples, a slight increase in bioaccessibility occurred for decreases in soil particle size. This deviation may be due to an increase in the surface area to volume ratio that accompanies reduced particle sizes. Investigation on extraction solution dilution effects indicated that bioaccessibility increased with increasing dilution ratio as found for the pure barite sample.

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APPENDIX A: DETAILED DATA TABLES

Table A.1: Barium Bioaccessibility for Barite using the Simplified Bioaccessibility Extraction Test (SBET) and the Sequential Stomach and Intestinal Phase Protocol (Gastrointestinal – GI) Protocol with Solution-to-Solid Ratio of 100:1

Sample ID	pH of Extract	Final Volume of Extract (L)	Barium Added (mg/kg)	Weight Use (g)	Barium in Extracts (mg/L)	Bioaccessibility (%)
SBET						
SBPH 15-1	1.5	0.100	576500	0.9881	7.70	0.135
SBPH 15-2	1.5	0.100	576500	0.9946	7.30	0.127
SBPH 15-3	1.5	0.100	576500	0.9951	7.30	0.127
					Mean	***0.130***
					Standard Dev	***0.005***
Gastrointestinal (GI) Model - Stomach Phase						
GIPH 20-1	1.86	0.100	588200	1.0082	2.80	0.047
GIPH 20-2	1.85	0.100	588200	1.0123	2.88	0.048
GIPH 20-3	1.86	0.100	588200	0.9912	2.82	0.048
					Mean	***0.048***
					Standard Dev	***0.001***
Gastrointestinal (GI) Model - Intestinal Phase						
GIPH 70-1	6.96	0.100	588200	1.0082	3.63	0.061
GIPH 70-2	6.9	0.105	588200	1.0123	3.76	0.066
GIPH 70-3	6.88	0.105	588200	0.9912	4.12	0.074
					Mean	***0.067***
					Standard Dev	***0.007***

Table A.2: Barium Bioaccessibility for NIST 2711 using the Simplified Bioaccessibility Extraction Test (SBET) and the Sequential Stomach and Intestinal Phase Protocol (Gastrointestinal – GI) Protocol with Solution-to-Solid Ratio of 100:1

Sample ID	pH of Extract	Final Volume of Extract (L)	Barium (mg/kg)	Weight use (g)	Barium in Extracts (mg/L)	Bioaccessibility (%)
SBET						
SBPH 15-4	1.5	0.100	726	0.9577	1.43	20.6
SBPH 15-5	1.5	0.100	726	1.0010	1.41	19.4
SBPH 15-6	1.5	0.100	726	1.0036	1.37	18.8
					Mean	***19.6***
					Std Dev	***0.89***
Gastrointestinal (GI) Model - Stomach Phase						
GIPH 20-4	1.64	0.100	726	1.0047	1.32	18.0
GIPH 20-5	1.95	0.100	726	0.9867	1.19	16.6
GIPH 20-6	1.92	0.100	726	0.9936	1.18	16.3
					Mean	***17.0***
					Std Dev	***0.94***
Gastrointestinal (GI) Model - Intestinal Phase						
GIPH 70-4	6.83	0.105	726	1.0047	0.72	10.4
GIPH 70-5	6.9	0.105	726	0.9867	0.73	10.7
GIPH 70-6	6.9	0.100	726	0.9936	0.62	8.6
					Mean	***9.9***
					Std Dev	***1.1***
Procedural Blank for GI Model						
GIPH 2	1.89	0.100	0	0	0.044	
GIPH 7	7	0.100	0	0	0.17	

Table A.3: Barium Bioaccessibility at Different pH using the Simplified Bioaccessibility Extraction Test (SBET) with Solution-to-Solid Ratio of 100:1

Sample ID	pH of Extract	Barium (mg/kg)	Weight use (g)	Barium in Extracts (mg/L)	Bioaccessibility (%)
SBPH 11-1	1.1	576500	0.9878	10.0	0.176
SBPH 11-2	1.1	576500	0.9853	10.7	0.188
SBPH 11-3	1.1	576500	1.0098	9.77	0.168
				Mean	***0.177***
				Std Dev	***0.010***
SBPH 15-1	1.5	576500	0.9881	7.70	0.135
SBPH 15-2	1.5	576500	0.9946	7.30	0.127
SBPH 15-3	1.5	576500	0.9951	7.30	0.127
				Mean	***0.130***
				Std Dev	***0.005***
SBPH 20-1	2.0	576500	0.9997	5.50	0.095
SBPH 20-2	2.0	576500	1.0026	3.42	0.059
SBPH 20-3	2.0	576500	0.9936	4.63	0.081
				Mean	***0.078***
				Std Dev	***0.018***
SBPH 30-1	3.0	576500	0.9864	3.38	0.059
SBPH 30-2	3.0	576500	1.0240	3.34	0.057
SBPH 30-3	3.0	576500	1.0073	3.41	0.059
				Mean	***0.058***
				Std Dev	***0.001***
SBPH 40-1	4.0	576500	1.0132	2.09	0.036
SBPH 40-2	4.0	576500	0.9991	2.10	0.036
SBPH 40-3	4.0	576500	1.0329	2.00	0.034
				Mean	***0.035***
				Std Dev	***0.002***

Table A.4: Effect of Extraction Solution-to-Solid Ratio on Barium Bioaccessibility for Pure Barite

Sample ID	Liquid to Solid ratio	Barium (mg/kg)	Weight used for extraction (g)	Barium conc in extract (mg/L)	Barium Bioaccessibility (%)
Dilution 1A	10,000:1	576400	0.0113	8.49	13.0
Dilution 1B	10,000:1	576400	0.0126	7.93	10.9
Dilution 1C	10,000:1	576400	0.0105	7.80	12.9
				Mean	***12.3***
				Std Dev	***1.2***
Dilution 5A	5,000:1	576400	0.0200	10.80	9.37
Dilution 5B	5,000:1	576400	0.0207	12.20	10.2
Dilution 5C	5,000:1	576400	0.0202	10.80	9.27
				Mean	***9.62***
				Std Dev	***0.52***
Dilution 2A	2,000:1	576400	0.0543	7.19	2.29
Dilution 2B	2,000:1	576400	0.0515	7.22	2.43
Dilution 2C	2,000:1	576400	0.0530	7.77	2.54
				Mean	***2.42***
				Std Dev	***0.12***
Dilution 3A	1,000:1	576400	0.1033	7.61	1.28
Dilution 3B	1,000:1	576400	0.1001	9.95	1.72
Dilution 3C	1,000:1	576400	0.1029	10.00	1.68
				Mean	***1.56***
				Std Dev	***0.24***
Dilution 4A	500:1	576400	0.2095	9.95	0.82
Dilution 4B	500:1	576400	0.2052	10.00	0.84
Dilution 4C	500:1	576400	0.2082	10.00	0.83
				Mean	***0.83***
				Std Dev	***0.01***
SBPH 15-1	100:1	576500	0.9881	7.70	0.135
SBPH 15-2	100:1	576500	0.9946	7.30	0.127
SBPH 15-3	100:1	576500	0.9951	7.30	0.127
				Mean	***0.130***
				Std Dev	***0.005***

Table A.5: Effect of Barite Particle Size on Barium Bioaccessibility using SBET with Solution-to-Solid Ratio of 1000:1

Sample ID	Sieve Size	Expected Barium conc (mg/kg)	Weight used for extraction (g)	Barium conc in extract (mg/L)	Barium Bioaccessibility (%)
BF1A	<250	576400	0.1004	10.10	1.74
BF1B	<250	576400	0.1182	9.89	1.45
BF1C	<250	576400	0.1056	10.10	1.65
				Mean	***1.61***
				Std Dev	***0.15***
BF2A	<125	576400	0.1254	10.20	1.41
BF2B	<125	576400	0.1037	9.98	1.67
BF2C	<125	576400	0.1030	10.10	1.70
				Mean	***1.59***
				Std Dev	***0.15***
BF3A	<63	576400	0.1035	11.50	1.92
BF3B	<63	576400	0.1006	10.60	1.82
BF3C	<63	576400	0.1002	10.40	1.80
				Mean	***1.85***
				Std Dev	***0.06***
BF4A	<45	576400	0.1066	10.20	1.66
BF4B	<45	576400	0.1068	10.20	1.65
BF5C	<45	576400	0.1011	10.00	1.71
				Mean	***1.67***
				Std Dev	***0.03***

Table A.6: Barium Bioaccessibility for Barite in OECD Soil using SBET with Solution-to-Solid Ratio of 1000:1

Sample ID	Weight used for extraction (g)	Barium in soil (mg/kg)	Barium recovery for NIST 2711 (%)	Barium recovery corrected (mg/kg)	Barium in extract (mg/L)	Barium Bioaccessibility (%)
OECD-Blank	1.0562	26.0	33.6	77	0.078	**9.5**
OECD-A	1.0561	3120	33.6	9286	11.7	11.9
OECD-B	1.0080	3040	33.6	9048	11.7	12.8
OECD-C	1.0064	3240	33.6	9643	11.6	12.0
					Mean	***12.2***
				Standard Deviation		***0.5***

(1 g added to 50g OECD soil)

Table A7: Barium Bioaccessibility for Site 3 Soil Samples using the SBET and Extraction Solution-to-Solid Ratio of 1000:1

Sample ID	SRM Recovery	Barium conc in soil (mg/kg)	Weight used for extraction (g)	Barium conc in extract (mg/L)	Barium Bioaccessibility (%)
<250 µm					
ST3-F1A	0.129	2210	0.1200	5.51	26.8
ST3-F1B	0.129	2170	0.1009	4.92	28.9
ST3-F1C	0.129	1660	0.1013	4.76	36.5
				Mean	***30.7***
				Std Dev	***5.0***
<125 µm					
ST3-F2A	0.129	1960	0.1013	5.29	34.3
ST3-F2B	0.129	2190	0.1095	5.62	30.2
ST3-F2C	0.129	1970	0.1000	5.65	36.9
				Mean	***33.8***
				Std Dev	***3.4***
<63 µm					
ST3-F3A	0.129	1750	0.1036	6.15	43.7
ST3-F3B	0.129	2050	0.1057	6.23	37.0
ST3-F3C	0.129	1980	0.1087	6.42	38.4
				Mean	***39.7***
				Std Dev	***3.5***
<45 µm					
ST3-F4A	0.129	1850	0.1080	6.87	44.3
ST3-F4B	0.129	1800	0.1003	6.70	47.8
ST3-F4C	0.129	1950	0.1008	6.67	43.7
				Mean	***45.3***
				Std Dev	***2.2***
Control Site, <250 µm					
ST3C-F1A	0.129	254	0.1060	0.167	8.00
ST3C-F1B	0.129	258	0.1211	0.192	7.92
ST3C-F1C	0.129	257	0.1018	0.161	7.93
				Mean	***7.95***
				Std Dev	***0.04***

Table A8: Barium Bioaccessibility for Site 4 Soil Samples using the SBET and Extraction Solution-to-Solid Ratio of 1000:1

Sample ID	SRM Recovery	Barium conc in soil (mg/kg)	Weight used for extraction (g)	Barium conc in extract (mg/L)	Barium Bioaccessibility (%)
<250 µm					
ST4-F1A	0.33	1720	0.1007	4.11	29.9
ST4-F1B	0.33	1550	0.1107	4.23	31.0
ST4-F1C	0.33	1300	0.1135	4.34	37.0
				Mean	***32.6***
				Std Dev	***3.8***
<125 µm					
ST4-F2A	0.33	1100	0.1045	4.94	54.1
ST4-F2B	0.33	1100	0.1274	5.37	48.2
ST4-F2C	0.33	1190	0.1103	5.18	49.7
				Mean	***50.7***
				Std Dev	***3.0***
<63 µm					
ST4-F3A	0.33	1210	0.1149	5.59	50.6
ST4-F3B	0.33	1340	0.1222	5.79	44.5
ST4-F3C	0.33	1230	0.1165	5.52	48.5
				Mean	***47.9***
				Std Dev	***3.1***
<45 µm					
ST4-F4A	0.33	1150	0.1209	5.87	53.1
ST4-F4B	0.33	1040	0.1060	5.66	64.6
ST4-F4C	0.33	1190	0.1205	6.07	53.3
				Mean	***57.0***
				Std Dev	***6.5***
Control Site, <250 µm					
ST4C-F1A	0.126	235	0.1113	0.186	8.96
ST4C-F1B	0.126	239	0.1052	0.191	9.57
ST4C-F1C	0.126	238	0.1131	0.193	9.03
				Mean	***9.18***
				Std Dev	***0.33***

Table A9: Total Metals, pH and Total Organic Carbon (TOC) in Site 4 Soil Samples

Particle Size	**<250 µm**			**<125 µm**		
Sample ID	ST4-F1A	ST4-F1B	ST4-F1C	ST4-F2A	ST4-F2B	ST4-F2C
pH	7.71	7.73	7.80	7.65	7.77	7.74
TOC (%C)	1.84	1.90	1.92	2.08	2.10	2.10
Total Metals (mg/kg)						
Antimony	<10	<10	<10	<10	<10	<10
Arsenic	6.6	7.1	6.8	7.0	7.6	6.1
Barium	1720	1550	1300	1100	1100	1190
Beryllium	0.72	0.75	0.73	0.75	0.75	0.78
Cadmium	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Chromium	20.7	21.0	20.9	21.9	21.2	22.1
Cobalt	9.2	9.3	9.1	9.4	9.5	9.3
Copper	32.2	32.7	33.7	39.7	42.4	41.4
Lead	<50	<50	<50	<50	<50	<50
Mercury	0.096	<0.050	0.063	0.076	0.081	0.077
Molybdenum	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0
Nickel	27.0	27.8	26.8	28.3	28.5	28.8
Selenium	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Silver	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Tin	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
Vanadium	34.0	30.4	33.7	35.1	33.1	33.3
Zinc	86.0	86.4	84.0	90.7	90.7	92.8

Table A9: Total Metals, pH and Total Organic Carbon (TOC) in Site 4 Soil Samples (Continued)

Particle Size	**<63 µm**			**<45 µm**		
Sample ID	ST4-F3A	ST4-F3B	ST4-F3C	ST4-F4A	ST4-F4B	ST4-F4C
pH	7.72	7.76	7.74	8.07	7.69	7.72
TOC (%C)	2.07	2.10	2.06	2.06	2.10	2.09
Total Metals (mg/kg)						
Antimony	<10	<10	<10	<10	<10	<10
Arsenic	6.0	6.6	6.6	6.5	7.1	7.2
Barium	1210	1340	1230	1150	1040	1190
Beryllium	0.79	0.84	0.83	0.84	0.82	0.81
Cadmium	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Chromium	22.5	23.4	23.6	24.8	23.3	22.5
Cobalt	9.4	9.5	9.5	9.7	9.7	9.6
Copper	55.2	54.8	56.3	53.9	53.9	51.7
Lead	<50	<50	<50	<50	<50	<50
Mercury	0.081	0.078	0.091	0.095	0.081	0.091
Molybdenum	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0
Nickel	28.6	28.9	29.1	30.3	30.6	30.0
Selenium	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Silver	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Tin	5.5	5.9	5.1	<5.0	<5.0	<5.0
Vanadium	34.0	37.8	35.3	38.9	35.2	34.9
Zinc	95.3	95.9	96.0	102	103	101

Table A10: Total Metals, pH and Total Organic Carbon (TOC) in Site 4 Control Soil Samples

Particle Size	**Control Site <250m**		
Sample ID	ST4C-F1A	ST4C-F1B	ST4C-F1C
pH	7.93	7.97	7.90
TOC (%C)	0.610	0.670	0.670
Total Metals (mg/kg)			
Antimony	<10	<10	<10
Arsenic	5.6	5.5	5.5
Barium	235	239	238
Beryllium	0.77	0.78	0.79
Cadmium	<0.50	<0.50	<0.50
Chromium	18.4	19.1	18.7
Cobalt	7.8	7.9	7.8
Copper	31.0	31.2	31.7
Lead	<50	<50	<50
Mercury	0.063	0.063	0.063
Molybdenum	<4.0	<4.0	<4.0
Nickel	24.6	25.2	24.6
Selenium	<2.0	<2.0	<2.0
Silver	<2.0	<2.0	<2.0
Tin	<5.0	<5.0	<5.0
Vanadium	29.5	32.4	32.7
Zinc	70.1	75.8	71.5

Table A.11: Barium Bioaccessibility for the <45 µm Site 2 Soil Samples using SBET and Different Extraction Solution-to-Solid Ratio

Sample ID	Liquid to soil ratio	SRM Recovery (%)	Barium conc (mg/kg)	Weight used for extraction (g)	Barium conc in extract (mg/L)	Barium Bioaccessibility (%)
S2-D1A	100:1	0.124	1062	0.9925	9.70	11.43
S2-D1B	100:1	0.124	1062	1.0032	9.79	11.41
S2-D1C	100:1	0.124	1062	1.0287	10.10	11.48
					Mean	***11.4***
					Std Dev	***0.036***
S2-D2A	1,000:1	0.124	1062	0.1035	11.30	127.7
S2-D2B	1,000:1	0.124	1062	0.1163	11.40	114.6
S2-D2C	1,000:1	0.124	1062	0.1001	11.40	133.2
					Mean	***125.1***
					Std Dev	***9.53***
S2-D3A	10,000:1	0.124	1062	0.0412	3.27	371.3
S2-D3B	10,000:1	0.124	1062	0.0422	3.27	362.5
S2-D3C	10,000:1	0.124	1062	0.0442	3.24	342.9
					Mean	***358.9***
					Std Dev	***14.5***

Table A.12: Comparison of Total Barium concentrations in Soil as Determined by XRF and ICP-OES

Sample ID	Barium concentration in <45 um fraction by ICP-OES (mg/kg)	Barium concentration in <38um fraction by XRF (mg/kg)*
Site 2	8,830	56,732
Site 3	14,570	11,537
Site 4	10,400	14,501

* Data provided by Exponent

Table A.13: Barium Bioaccessibility for Site 2 Soil Samples Using Barium Concentrations as Determined by XRF

Sample ID	Liquid to Soil ratio	Barium in <38 µm fraction using XRF (mg/kg)	Weight used for extraction (g)	Barium in extract (mg/L)	Barium Bioaccessibility (%)
S2-D1A	100:1	56732	0.9925	9.70	1.72
S2-D1B	100:1	56732	1.0032	9.79	1.72
S2-D1C	100:1	56732	1.0287	10.10	1.73
				Mean	***1.72***
				Std Dev	***0.005***
S2-D2A	1,000:1	56732	0.1035	11.30	19.2
S2-D2B	1,000:1	56732	0.1163	11.40	17.2
S2-D2C	1,000:1	56732	0.1001	11.40	20.0
				Mean	***18.8***
				Std Dev	***1.4***
S2-D3A	10,000:1	56732	0.0412	3.27	55.9
S2-D3B	10,000:1	56732	0.0422	3.27	54.6
S2-D3C	10,000:1	56732	0.0442	3.24	51.6
				Mean	***54.1***
				Std Dev	***2.1***

Table A.14: Barium Bioaccessibility for the <45 µm Site 3 Soil Samples using SBET and Different Extraction Solution-to-Solid Ratio

Sample ID	Liquid to Solid ratio	SRM Recovery (%)	Barium conc (mg/kg)	Weight used for extraction (g)	Barium conc in extract (mg/L)	Barium Bioaccessibility (%)
S3-D1A	100:1	0.129	1867	1.0050	11.90	8.18
S3-D1B	100:1	0.129	1867	1.0012	11.90	8.21
S3-D1C	100:1	0.129	1867	1.0074	11.50	7.88
					Mean	***8.09***
					Std Dev	***0.18***
S3-D2A	1,000:1	0.129	1867	0.1022	6.71	45.4
S3-D2B	1,000:1	0.129	1867	0.1003	6.42	44.2
S3-D2C	1,000:1	0.129	1867	0.1009	6.77	46.37
					Mean	***45.3***
					Std Dev	***1.1***
S3-D3A	10,000:1	0.129	1867	0.0425	0.79	51.2
S3-D3B	10,000:1	0.129	1867	0.0412	0.94	63.2
S3-D3C	10,000:1	0.129	1867	0.0431	0.52	33.6
					Mean	***49.3***
					Std Dev	***14.8***

Table A.15: Barium Bioaccessibility for the <45 µm Site 5 Soil Samples using SBET and Different Extraction Solution-to-Solid Ratio

Sample ID	Liquid to Solid ratio	SRM recovery (%)	Barium conc (mg/kg)	Weight used for extraction (g)	Barium conc in extract (mg/L)	Barium Bioaccessibility (%)
S5-D1A	100:1	0.124	1443	1.0192	7.58	6.38
S5-D1B	100:1	0.124	1443	1.0072	8.07	6.88
S5-D1C	100:1	0.124	1443	1.0111	8.02	6.81
					Mean	***6.69***
					Standard Dev	***0.26***
S5-D2A	1,000:1	0.124	1443	0.1006	2.91	24.8
S5-D2B	1,000:1	0.124	1443	0.1038	3.11	25.7
S5-D2C	1,000:1	0.124	1443	0.1151	3.40	25.3
					Mean	***25.3***
					Standard Dev	***0.45***
S5-D3A	10,000:1	0.124	1443	0.0433	0.38	30.4
S5-D3B	10,000:1	0.124	1443	0.0435	0.43	33.9
S5-D3C	10,000:1	0.124	1443	0.0420	0.43	34.8
					Mean	***33.0***
					Standard Dev	***2.3***

APPENDIX B: LABORATORY BATCH SUMMARIES AND SAMPLE PREPARATION

Table B.1: Batch Summary for SBET Extraction at Variable pH (1 – 2)

Date of Extraction:	08-Sep-05
Start Time:	12:05PM
Stop Time:	1:05PM
Initial temperature of water bath (^{o}C)	38.2
Final temperature of water bath (^{o}C)	38.2

Sample ID	Weight used (g)	Initial pH	Final pH	Comments
SBPH 11-1	0.9904	1.08	1.13	
SBPH 11-2	0.9884	1.09	1.13	
SBPH 11-3	1.0098	1.08	1.13	
SBPH 11-4	---	1.09	1.14	Blank
SBPH 15-1	0.9881	1.48	1.54	
SBPH 15-2	0.9946	1.50	1.55	
SBPH 15-3	0.9951	1.51	1.55	
SBPH 15-4	0.9577	1.56	1.62	SRM NIST 2711
SBPH 20-1	0.9997	2.03	2.09	
SBPH 20-2	1.0020	2.03	2.08	

Table B.2: Batch Summary for SBET Extraction at Variable pH (2 – 4)

Date of Extraction:	08-Sep-05
Start Time	2:53pm
Stop Time	3:53pm
Initial temperature of water bath (^{o}C)	38.4
Final temperature of water bath (^{o}C)	38.2

Sample ID	Weight Used (g)	Initial pH	Final pH	Comments
SBPH 20-3	0.9936	2.09	2.01	
SBPH 30-1	0.9864	3.12	3.08	
SBPH 30-2	1.0244	3.07	3.06	
SBPH 30-3	1.0073	3.09	3.04	
SBPH 40-1	1.0132	4.31	4.07	
SBPH 40-2	0.9991	4.26	4.07	
SBPH 40-3	1.0329	4.25	4.06	
SBPH 40-4	---	4.19	4.04	Blank

Table B.3: Batch Summary for Gastrointestinal (GI) Extraction Model

Date of Extraction:			09-Sep-05		
Start Time:			11:05AM		
Stop Time:			1:05PM		
Initial temperature of water bath (°C)			38.0		
Final temperature of water bath (°C)			38.2		
Sample ID	**Weight (g)**	**pH at 5min stomach phase**	**pH after adjustment for 1 hr intestinal phase**	**Final pH after 3 hr intestinal phase extraction**	**Comments**
GI-1	1.0082	1.86	6.96	7.42	Barite
GI-2	1.0123	1.85	6.90	7.43	Barite
GI-3	0.9912	1.86	6.88	7.37	Barite
GI-4	1.0047	1.64	6.83	7.27	SRM NIST 2711
GI-5	0.9867	1.95	6.90	7.37	SRM NIST 2711
GI-6	0.9936	1.92	7.0	7.42	SRM NIST 2711
GI-7	--	1.89	7.0	7.58	Blank
GI-8	--	1.89	7.0	7.58	Blank

Table B.4: Batch Summary for SBET Extraction for Different Particle Size Fraction for Pure Barite

Date of Extraction:				24-Nov-05	
Start Time:				10.20 AM	
Stop Time:				11.20 AM	
Initial temperature of water bath (°C)				36.8	
Final temperature of water bath (°C)				37.4	
Sample ID	**Sieve Size**	**Weight (g)**	**Initial pH**	**Final pH**	**Comments**
BF1A	<250	0.1004	1.61	1.62	
BF1B	<250	0.1182	1.60	1.62	
BF1C	<250	0.1056	1.60	1.61	
BF2A	<125	0.1254	1.60	1.62	
BF2B	<125	0.1037	1.60	1.62	
BF2C	<125	0.1030	1.60	1.62	
BF4A	<45	0.1066	1.60	1.62	
BF4B	<45	0.1068	1.60	1.62	
BF4C	<45	0.1011	1.60	1.61	
BL05-13	--	1.0561	1.60	1.63	Blank

Table B.5: Batch Summary for SBET Extraction for OECD Soil and <63 µm Particle Size Fraction for Pure Barite

Date of Extraction:	24-Nov-05
Start Time:	2:054PM
Stop Time:	3:05 PM
Initial temperature of water bath (^{o}C)	37.4
Final temperature of water bath (^{o}C)	37.5

Sample ID	Sieve Size	Weight (g)	Initial pH	Final pH	Comments
BF3A	**<63**	0.1035	1.62	1.63	
BF3B	**<63**	0.1006	1.62	1.61	
BF3C	**<63**	0.1002	1.62	1.59	
OECD-A		1.0561	1.46	1.46	Spiked OECD soil
OECD-B		1.0080	1.44	1.46	Spiked OECD soil
OECD-C		1.0064	1.48	1.47	Spiked OECD soil
OECD-D		1.0562	1.48	1.47	Blank OECD soil
SR05-13		1.0036	1.62	1.62	SRM

Table B.6: Batch Summary for SBET Extraction for Pure Barite at Different Dilution Ratios

Date of Extraction:	14-Nov-05
Start Time:	10:20 PM
Stop Time:	11:20 PM
Initial temperature of water bath (^{o}C)	38.0
Final temperature of water bath (^{o}C)	37.8

Sample ID	Dilution factor	Weight used (g)	Initial pH	Final pH	Comments
DIL 1A	10,000	0.0113	1.54	1.57	
DIL 1B	10,000	0.0126	1.54	1.57	
DIL 1C	10,000	0.0105	1.54	1.57	
DIL 2A	2000	0.0543	1.54	1.57	
DIL 2B	2000	0.0515	1.54	1.58	
DIL 2C	2000	0.0530	1.54	1.58	
DIL 3A	1000	0.1033	1.54	1.58	
DIL 3B	1000	0.1001	1.54	1.58	
DIL 3C	1000	0.1039	1.54	1.58	
BL05-12	--	---	1.54	1.57	blank

Table B.7: Batch Summary for SBET Extraction for Pure Barite at Two Additional Dilution Ratios

Date of Extraction:	14-Nov-05
Start Time:	10:20 PM
Stop Time:	11:20 PM
Initial temperature of water bath (°C)	38.0
Final temperature of water bath (°C)	37.8

Sample ID	Dilution factor	Weight used (g)	Initial pH	Final pH	Comments
DIL 4A	500	0.2095	1.55	1.53	
DIL 4B	500	0.2052	1.55	1.54	
DIL 4C	500	0.2082	1.55	1.54	
DIL 5A	5000	0.0200	1.55	1.54	
DIL 5B	5000	0.0207	1.55	1.55	
DIL 5C	5000	0.0202	1.55	1.55	
SR05-12	100	1.001	1.62	1.59	SRM

Table B.8: Laboratory Data for Test Soil Sample Drying at <40°C

Sample ID	Bowl (g)	Bowl & Wet Soil (g)	Wet soil (g)	Bowl & Dry Soil (g)	Dry Soil (g)	Moisture (g)	Moisture (%)
Site 2							
ST2-1	176.2	1023.1	846.9	942.2	766.0	80.9	9.55
ST2-2	209.2	1114.5	905.3	1029	819.8	85.5	9.44
ST2-3	158.1	892.0	733.9	819.9	661.8	72.1	9.82
						Mean	***9.61***
						Std Dev	***0.20***
Site 3							
ST3-1	158.6	713.8	700.5	722.7	564.1	136.4	19.47
ST3-2	209.2	805.6	804.7	850.3	641.1	163.6	20.33
ST3-3	175.8	770.4	689.1	715.4	539.6	149.5	21.69
ST3-4	157.7	724.4	632.0	669.0	511.3	120.7	19.10
						Mean	***20.15***
						Std Dev	***1.15***
Control							
ST3-C1	159.67	698.2	776.2	779.2	619.53	156.67	20.18
ST3-C2	160.5	782.9	722.2	746.5	586	136.2	18.86
						Mean	***19.52***
Site 4							
ST4-1	175.8	713.8	538.0	622.8	447.0	91.0	16.91
ST4-2	209.2	805.6	596.4	705.4	496.2	100.2	16.80
ST4-3	158.5	770.4	611.9	644.1	485.6	126.3	20.64
						Mean	***18.12***
						Std Dev	***2.18***
Control							
ST4-C1	158	698.2	540.2	619.3	461.3	78.9	14.61
ST4-C2	158.6	782.9	624.3	688.2	529.6	94.7	15.17
						Mean	***14.89***
Site 5							
ST5-1	157.7	851.7	694.0	687.1	529.4	164.6	23.72
ST5-2	160.7	790.9	630.2	639	478.3	151.9	24.10
ST5-3	158.6	835.8	677.2	678.7	520.1	157.1	23.20
ST5-4	158.6	836.4	677.8	675.5	516.9	160.9	23.74
						Mean	***23.47***
						Std Dev	***0.38***
Control							
ST5-1	159.9	1120.2	960.3	951.6	791.7	168.6	17.56

Table B.9: Laboratory Data for Test Soil Sample Sieving

Sample Labels	Sieve Size	Pan (g)	Pan & Sieve (g)	Pan, Sieve & Soil (g)	Pan & Sieved Soil (g)	Soil Used (g)	Sieved Soil (g)
Site 2							
ST2-F1	250µm	383.1	702.9	1237.1	461.2	534.2	78.1
ST2-F2	125µm	372.4	680.5	1343.4	446	662.9	73.6
ST2-F3	63µm	383.2	728.9	1265.1	430.1	536.2	46.9
ST2-F4	45µm	381.5	697.1	1188.4	416.6	491.3	35.1
Site 3							
ST3-F1	250µm	382.95	703.9	1229.0	520.0	525.1	137.0
ST3-F2	125µm	372.59	680.7	1219.5	484.3	538.8	111.7
ST3-F3	63µm	383.2	729	1219.8	481.1	490.8	97.9
ST3-F4	45µm	381.6	697	1430.3	488.9	733.3	107.3
Site 4							
ST4-F1	250µm	383.1	740	1693.9	649.3	953.9	266.2
ST4-F2	125µm	383.1	691.2	1177.7	498.4	486.5	115.3
ST4-F3	63µm	381.5	727.3	1228.6	464.4	501.3	82.9
ST4-F4	45µm	372.48	687.9	1156.3	445.6	468.4	73.1
Site 5							
ST5-F1	250µm	382.9	703.8	1345.3	478.3	641.5	95.4
ST5-F2	125µm	372.4	680.4	1198.2	465.7	517.8	93.3
ST5-F3	63µm	379.1	721.1				
ST5-F4	45µm	381.6	727.7				

Table B.10: Batch Summary for Site 3 Particle Size Fraction Extraction (1)

Date of Extraction:	
Start Time:	12:05PM
Stop Time:	1:05PM
Initial temperature of water bath OC:	36.8
Final temperature of water bath (OC):	37.2

Sample ID	Weight (g)	Initial pH	Final pH	Comments
ST3C-F1A	0.106	1.50	1.58	Control site, <250 µm
ST3C-F1B	0.1211	1.50	1.58	Control site, <250 µm
ST3C-F1C	0.1018	1.50	1.58	Control site, <250 µm
ST3-F1A	0.1200	1.50	1.58	<250 µm
ST3-F1B	0.1009	1.50	1.58	<250 µm
ST3-F1C	0.1013	1.50	1.58	<250 µm
ST3-F2A	0.1031	1.50	1.58	<125 µm
ST3-F2B	0.1095	1.50	1.58	<125 µm
ST3-F2C	0.1000	1.50	1.58	<125 µm
BL05-15	----	1.50	1.57	blank

Table B.11: Batch Summary for Site 3 Particle Size Fraction Extraction (2)

Date of Extraction:	
Start Time:	2:05PM
Stop Time:	305PM
Initial temperature of water bath OC:	36.8
Final temperature of water bath (OC):	37.2

Sample ID	Weight (g)	Initial pH	Final pH	Comments
ST3-F3A	0.1036	1.50	1.57	<63 µm
ST3-F3B	0.1057	1.50	1.57	<63 µm
ST3-F3C	0.1087	1.50	1.57	<63 µm
ST3-F4A	0.1080	1.50	1.59	<45 µm
ST3-F4B	0.1003	1.50	1.58	<45 µm
ST3-F4C	0.1008	1.50	1.57	<45 µm
SR05-15	1.0096	1.50	1.59	SRM

Table B.12 Batch Summary for Site 4 Particle Size Faction Extraction (1)

Date of Extraction:				
Start Time:		11:50 AM		
Stop Time:		12:50 PM		
Initial temperature of water bath (°C)		36.8		
Final temperature of water bath (°C)		37.2		
Sample ID	**Weight (g)**	**Initial pH**	**Final pH**	**Comments**
ST4C-F1A	0.1113	1.50	1.50	Control site, <250 µm
ST4C-F1B	0.1052	1.48	1.48	Control site, <250 µm
ST4C-F1C	0.1131	1.49	1.48	Control site, <250 µm
ST4-F1A	0.1007	1.50	1.49	<250 µm
ST4-F1B	0.1107	1.50	1.50	<250 µm
ST4-F1C	0.1138	1.49	1.50	<250 µm
ST4-F2A	0.1045	1.50	1.49	<125 µm
ST4-F2B	0.1284	1.50	1.51	<125 µm
BL05-21	-----	1.49	1.49	blank
SR05-21	0.1130	1.49	1.48	SRM

Table B.13: Batch Summary for Site 4 Particle Size Faction Extraction (2)

Date of Extraction:				
Start Time:		2:30 PM		
Stop Time:		3:30 PM		
Initial temperature of water bath (°C)		37.0		
Final temperature of water bath (°C)		37.2		
Sample ID	**Weight (g)**	**Initial pH**	**Final pH**	**Comments**
ST4-F2C	0.1149	1.47	1.50	<63 µm
ST4-F3A	0.1149	1.50	1.48	<63 µm
ST4-F3B	0.1222	1.48	1.49	<63 µm
ST4-F3C	0.1165	1.48	1.49	<63 µm
ST4-F4A	0.1209	1.46	1.48	<45 µm
ST4-F4B	0.1000	1.48	1.49	<45 µm
ST4-F4C	0.1205	1.48	1.49	<45 µm

Table B.14: Batch Summary for Site 2 Extraction at Different Dilution Ratios

Date of Extraction:			Sept 6, 2006	
Start Time:			12:20 PM	
Stop Time:			1:20 PM	
Initial temperature of water bath (°C)			36.0	
Final temperature of water bath (°C)			36.0	
Sample ID	**Weight (g)**	**Initial pH**	**Final pH**	**Fluid to soil ratio**
S2D1A	0.9925	1.50	1.74	100:1
S2D1B	1.0032	1.50	1.73	100:1
S2D1C	1.0287	1.49	1.59	100:1
S2D2A	0.1035	1.50	1.59	1000:1
S2D2B	0.1163	1.50	1.58	1000:1
S2D2C	0.1001	1.49	1.58	1000:1
S2D3A	0.0412	1.50	1.63	10000:1
S2D3B	0.0422	1.50	1.62	10000:1
S2D3C	0.0442	1.50	1.57	10000:1
BL017	-----	1.50	1.57	blank

Table B.15: Batch Summary for Site 3 Extraction at Different Dilution Ratios

Date of Extraction:				
Start Time:				
Stop Time:				
Initial temperature of water bath (°C)			370	
Final temperature of water bath (°C)			370	
Sample ID	**Weight (g)**	**Initial pH**	**Final pH**	**Fluid to soil ratio**
S3D1A	1.0050	1.50	1.56	100:1
S3D1B	1.0012	1.50	1.57	100:1
S3D1C	1.0074	1.50	1.62	100:1
S3D2A	0.1022	1.50	1.62	1000:1
S3D2B	0.1003	1.50	1.63	1000:1
S3D2C	0.1009	1.50	1.63	1000:1
S3D3A	0.0425	1.50	1.56	10000:1
S3D3B	0.0412	1.50	1.59	10000:1
S3D3C	0.0431	1.50	1.57	10000:1

Table B.16: Batch Summary for Site 4 Extraction at Different Dilution Ratios

Date of Extraction:	Sept 6, 2006
Start Time:	12:20 PM
Stop Time:	1:20 PM
Initial temperature of water bath (°C)	36.0
Final temperature of water bath (°C)	36.0

Sample ID	Weight (g)	Initial pH	Final pH	Fluid to soil ratio
S4D1A	0.9989	1.50	1.72	100:1
S4D1B	1.0105	1.50	1.73	100:1
S4D1C	1.0014	1.50	1.74	100:1
S4D2A	0.1016	1.50	1.64	1000:1
S4D2B	0.1000	1.50	1.61	1000:1
S4D2C	0.1044	1.50	1.61	1000:1
S4D3A	0.0424	1.50	1.66	10000:1
S4D3B	0.0410	1.50	1.63	10000:1
S4D3C	0.0423	1.50	1.65	10000:1

Table B.17: Batch Summary for Site 2Extraction at Different Dilution Ratios

Date of Extraction:	Sept 6, 2006
Start Time:	12:20 PM
Stop Time:	1:20 PM
Initial temperature of water bath (°C)	36.0
Final temperature of water bath (°C)	36.0

Sample ID	Weight (g)	Initial pH	Final pH	Fluid to soil ratio
S4D1A	1.0192	1.50	1.63	100:1
S4D1B	1.0072	1.50	1.61	100:1
S4D1C	1.0111	1.49	1.61	100:1
S4D2A	0.1006	1.50	1.52	1000:1
S4D2B	0.1038	1.50	1.50	1000:1
S4D3C	0.1151	1.49	1.50	1000:1
S4D3A	0.0433	1.50	1.63	10000:1
S4D3B	0.0435	1.50	1.58	10000:1
S4D3C	0.0420	1.50	1.57	10000:1
SR017	1.0230	1.50	1.64	SRM

Table B.18: Sample Air Drying for Alberta Environment $BACl_2$ Extraction

Sample ID	Bowl (g)	Bowl & wet soil (g)	Wet soil (g)	Bowl & Dry Soil (g)	Dry Soil (g)
Site 2	158.4	1006.4	848.0	936.4	778.0
Site 3	209.2	965.4	756.2	832.6	623.4
Site 4	175.7	919.3	743.6	806.9	631.2
Site 5	157.8	720.0	562.2	597.4	439.6

APPENDIX C: STATISTICAL ANALYSIS

C.1: ONE-WAY ANOVA OF BARIUM BIOACCESSIBILITY VERSUS PARTICLE SIZE FRACTION FOR PURE BARITE

```
Analysis of Variance for % bioaccessibility
Source     DF        SS        MS        F        P
particle    3    0.1222    0.0407     3.04    0.093
Error       8    0.1072    0.0134
Total      11    0.2294
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  -+---------+---------+---------+-----
 45         3    1.6777    0.0332        (---------*--------)
 63         3    1.8523    0.0669                   (---------*--------)
125         3    1.5940    0.1592   (---------*--------)
250         3    1.6187    0.1506     (--------*---------)
                                   -+---------+---------+---------+-----
Pooled StDev =    0.1158          1.44      1.60      1.76      1.92

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0126

Critical value = 4.53

Intervals for (column level mean) - (row level mean)

                45          63         125
       63     -0.4775
               0.1281
      125     -0.2191     -0.0445
               0.3865      0.5611
      250     -0.2438     -0.0691     -0.3275
               0.3618      0.5365      0.2781
```

C.2: ONE-WAY ANOVA OF BARIUM BIOACCESSIBILITY VERSUS SOIL PARTICLE SIZE FRACTION FOR SITE 3 SOIL SAMPLES

```
Source         DF      SS     MS     F      P
Particle Size   3  375.2  125.1  9.11  0.006
Error           8  109.8   13.7
Total          11  485.0

S = 3.705   R-Sq = 77.36%   R-Sq(adj) = 68.87%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
 45    3  45.334  2.218                       (------*------)
 63    3  39.776  3.519               (------*------)
125    3  33.867  3.411      (------*------)
250    3  30.768  5.096  (------*------)
                         ---+---------+---------+---------+------
                         28.0      35.0      42.0      49.0

Pooled StDev = 3.705


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Particle Size

Individual confidence level = 98.74%

Particle Size =  45 subtracted from:

Particle
Size        Lower   Center   Upper    +---------+---------+---------+---------
 63       -15.248   -5.558   4.131           (-------*-------)
125       -21.157  -11.468  -1.778      (-------*--------)
250       -24.256  -14.566  -4.877    (-------*-------)
                                      +---------+---------+---------+---------
                                    -24       -12         0        12

Particle Size =  63 subtracted from:

Particle
Size        Lower  Center  Upper    +---------+---------+---------+---------
125       -15.599  -5.909  3.780           (-------*-------)
250       -18.697  -9.008  0.682        (-------*--------)
                                    +---------+---------+---------+---------
                                  -24       -12         0        12

Particle Size = 125 subtracted from:

Particle
Size        Lower  Center  Upper    +---------+---------+---------+---------
250       -12.788  -3.098  6.591             (-------*-------)
                                    +---------+---------+---------+---------
                                  -24       -12         0        12
```

C.3: ONE-WAY ANOVA OF BARIUM BIOACCESSIBILITY VERSUS SOIL PARTICLE SIZE FRACTION FOR SITE 4 SOIL SAMPLES

```
Source         DF      SS     MS      F      P
Particle Size   3   964.1  321.4  16.64  0.001
Error           8   154.5   19.3
Total          11  1118.6

S = 4.395   R-Sq = 86.19%   R-Sq(adj) = 81.01%


                               Individual 95% CIs For Mean Based on
                               Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
 45    3  57.075  6.597                          (-----*-----)
 63    3  47.917  3.101               (-----*-----)
125    3  50.719  3.057                   (-----*-----)
250    3  32.674  3.843  (-----*-----)
                         ---+---------+---------+---------+------
                           30        40        50        60

Pooled StDev = 4.395


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Particle Size

Individual confidence level = 98.74%


Particle Size =  45 subtracted from:

Particle
Size      Lower   Center    Upper  --------+---------+---------+---------+-
 63     -20.652   -9.158    2.336          (----*-----)
125     -17.850   -6.357    5.137           (-----*-----)
250     -35.895  -24.401  -12.908  (-----*-----)
                                   --------+---------+---------+---------+-
                                         -20         0        20        40


Particle Size =  63 subtracted from:

Particle
Size      Lower   Center   Upper  --------+---------+---------+---------+-
125      -8.692    2.801  14.295                (----*-----)
250     -26.737  -15.243  -3.750       (----*-----)
                                  --------+---------+---------+---------+-
                                        -20         0        20        40


Particle Size = 125 subtracted from:

Particle
Size      Lower   Center   Upper  --------+---------+---------+---------+-
250     -29.538  -18.045  -6.551     (-----*-----)
                                  --------+---------+---------+---------+-
                                        -20         0        20        40
```

C.4: ONE-WAY ANOVA OF TOTAL BARIUM VERSUS SOIL PARTICLE SIZE FRACTION FOR SITE 3 SOIL SAMPLES

```
Source         DF      SS     MS     F      P
particle size   3   57167  19056  0.54  0.669
Error           8  282800  35350
Total          11  339967

S = 188.0   R-Sq = 16.82%   R-Sq(adj) = 0.00%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
 45    3  1866.7   76.4  (-----------*------------)
 63    3  1926.7  157.0     (-----------*------------)
125    3  2040.0  130.0           (-----------*------------)
250    3  2013.3  306.6         (------------*-----------)
                         ---------+---------+---------+---------+
                              1800      2000      2200      2400

Pooled StDev = 188.0


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of particle size

Individual confidence level = 98.74%


particle size =  45 subtracted from:

particle
size      Lower  Center  Upper  -------+---------+---------+---------+--
 63      -431.7    60.0  551.7       (-------------*-------------)
125      -318.4   173.3  665.1          (-------------*-------------)
250      -345.1   146.7  638.4         (-------------*-------------)
                                -------+---------+---------+---------+--
                                     -350         0       350       700


particle size =  63 subtracted from:

particle
size      Lower  Center  Upper  -------+---------+---------+---------+--
125      -378.4   113.3  605.1        (-------------*-------------)
250      -405.1    86.7  578.4       (-------------*--------------)
                                -------+---------+---------+---------+--
                                     -350         0       350       700


particle size = 125 subtracted from:

particle
size      Lower  Center  Upper  -------+---------+---------+---------+--
250      -518.4   -26.7  465.1    (-------------*-------------)
                                -------+---------+---------+---------+--
                                     -350         0       350       700
```

C.5: One-way ANOVA of Total Barium versus Soil Particle Size Fraction for Site 4 Soil Samples

```
Source         DF      SS      MS     F      P
Particle Size   3  312067  104022  7.14  0.012
Error           8  116533   14567
Total          11  428600

S = 120.7   R-Sq = 72.81%   R-Sq(adj) = 62.61%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev   --+---------+---------+---------+-------
 45    3  1126.7   77.7   (-------*-------)
 63    3  1260.0   70.0          (-------*-------)
125    3  1130.0   52.0   (--------*-------)
250    3  1523.3  211.3                       (-------*-------)
                          --+---------+---------+---------+-------
                         1000      1200      1400      1600

Pooled StDev = 120.7


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Particle Size

Individual confidence level = 98.74%


Particle Size =  45 subtracted from:

Particle
Size       Lower  Center  Upper  --------+---------+---------+---------+-
 63       -182.3   133.3  449.0               (-------*-------)
125       -312.3     3.3  319.0            (-------*-------)
250         81.0   396.7  712.3                       (-------*-------)
                                 --------+---------+---------+---------+-
                                      -400         0       400       800


Particle Size =  63 subtracted from:

Particle
Size       Lower  Center  Upper  --------+---------+---------+---------+-
125       -445.7  -130.0  185.7         (-------*-------)
250        -52.3   263.3  579.0                   (-------*------)
                                 --------+---------+---------+---------+-
                                      -400         0       400       800


Particle Size = 125 subtracted from:

Particle
Size      Lower  Center  Upper  --------+---------+---------+---------+-
250        77.7   393.3  709.0                      (-------*-------)
                                --------+---------+---------+---------+-
                                     -400         0       400       800
```

C.6: Correlations Analysis for Soil Particle Size Fractions, Bioaccessibility, pH, TOC and Total Metals Content for Site 4

```
             Particle Siz    Bioacces          As          Ba
Bioaccess          -0.855
                    0.000

As                  0.125       0.048
                    0.699       0.882

Ba                  0.718      -0.907      -0.147
                    0.009       0.000       0.649

Be                 -0.874       0.616      -0.223      -0.459
                    0.000       0.033       0.486       0.134

Cr                 -0.845       0.649      -0.292      -0.501
                    0.001       0.022       0.357       0.097

Co                 -0.865       0.777       0.224      -0.614
                    0.000       0.003       0.484       0.034

Cu                 -0.949       0.714      -0.229      -0.568
                    0.000       0.009       0.474       0.054

Hg                 -0.543       0.369      -0.185      -0.156
                    0.068       0.238       0.565       0.628

Ni                 -0.889       0.840       0.056      -0.660
                    0.000       0.001       0.862       0.019

V                  -0.657       0.470      -0.233      -0.344
                    0.020       0.123       0.465       0.273

Zn                 -0.929       0.825      -0.062      -0.612
                    0.000       0.001       0.849       0.034

pH                 -0.179       0.031      -0.175      -0.114
                    0.579       0.924       0.587       0.725

TOC                -0.880       0.866       0.004      -0.866
                    0.000       0.000       0.990       0.000

Cell Contents: Pearson correlation
               P-Value
```

C.6: CONTINUED

```
                     Be          Cr          Co          Cu
Cr                0.946
                  0.000

Co                0.805       0.800
                  0.002       0.002

Cu                0.923       0.875       0.797
                  0.000       0.000       0.002

Hg                0.406       0.490       0.481       0.510
                  0.190       0.106       0.113       0.090

Ni                0.846       0.827       0.949       0.808
                  0.001       0.001       0.000       0.001

V                 0.710       0.816       0.595       0.663
                  0.010       0.001       0.041       0.019

Zn                0.884       0.866       0.927       0.882
                  0.000       0.000       0.000       0.000

pH                0.360       0.517       0.307       0.218
                  0.250       0.085       0.332       0.495

TOC               0.665       0.615       0.703       0.743
                  0.018       0.033       0.011       0.006


                     Hg          Ni           V          Zn
Ni                0.444
                  0.148

V                 0.627       0.552
                  0.029       0.063

Zn                0.546       0.976       0.646
                  0.066       0.000       0.023

pH                0.247       0.278       0.537       0.278
                  0.439       0.382       0.072       0.382

TOC               0.281       0.745       0.446       0.723
                  0.376       0.005       0.146       0.008


                     pH
TOC               0.025
                  0.938

Cell Contents: Pearson correlation
               P-Value
```

APPENDIX D: LABORATORY REPORTS FOR ICP-OES ANALYSES

ALS Environmental

ALS

CERTIFICATE OF ANALYSIS

Date: September 28, 2005

ALS File No. W4351

Report On: PBET Water Analysis

Report To: **Royal Roads University**
Applied Research Division
2005 Sooke Road
Victoria, BC
V9B 5Y2

Attention: **Dr. Matthew Dodd**

Received: September 13, 2005

ALS ENVIRONMENTAL
per:

Can Dang, B.Sc. - Project Chemist
Andre Langlais, M.Sc. - Project Chemist

ALS CANADA LTD.
1988 Triumph Street, Vancouver, BC Canada V5L 1K5
Phone: 604-253-4188 Fax: 604-253-6700 Website: www.alsenviro.com

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File No. W4351

RESULTS OF ANALYSIS

	Barium T-Ba
Water	
SBPH 11-1 *ALS ID 1*	10.0
SBPH 11-2 *ALS ID 2*	10.7
SBPH 11-3 *ALS ID 3*	9.77
SBPH 11-4 *ALS ID 4*	<0.010
SBPH 15-1 *ALS ID 5*	7.70
SBPH 15-2 *ALS ID 6*	7.30
SBPH 15-3 *ALS ID 7*	7.30
SBPH 15-4 *ALS ID 8*	1.43
SBPH 20-1 *ALS ID 9*	5.50
SBPH 20-2 *ALS ID 10*	3.42
SBPH 20-3 *ALS ID 11*	4.63
SBPH 30-1 *ALS ID 12*	3.38
SBPH 30-2 *ALS ID 13*	3.34
SBPH 30-3 *ALS ID 14*	3.41
SBPH 40-1 *ALS ID 15*	2.09
SBPH 40-2 *ALS ID 16*	2.10
SBPH 40-3 *ALS ID 17*	2.00
SBPH 40-4 *ALS ID 18*	0.198
GIPH 20-1 *ALS ID 19*	2.84
GIPH 20-2 *ALS ID 20*	2.92

Results are expressed as milligrams per litre except where noted.
< = Less than the detection limit indicated.

RESULTS OF ANALYSIS

	Barium T-Ba
Water	
GIPH 20-3 *ALS ID 21*	2.86
GIPH 20-4 *ALS ID 22*	1.36
GIPH 20-5 *ALS ID 23*	1.23
GIPH 20-6 *ALS ID 24*	1.22
GIPH 20-7 *ALS ID 25*	0.044
GIPH 70-1 *ALS ID 26*	3.80
GIPH 70-2 *ALS ID 27*	3.93
GIPH 70-3 *ALS ID 28*	4.29
GIPH 70-4 *ALS ID 29*	0.89
GIPH 70-5 *ALS ID 30*	0.90
GIPH 70-6 *ALS ID 31*	0.79
GIPH 70-7 *ALS ID 32*	0.17

Results are expressed as milligrams per litre except where noted.
< = Less than the detection limit indicated.

Appendix 1 - METHODOLOGY

Outlines of the methodologies utilized for the analysis of the samples submitted are as follows

Metals in Water

This analysis is carried out using procedures adapted from "Standard Methods for the Examination of Water and Wastewater" 20th Edition 1998 published by the American Public Health Association, and with procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846 published by the United States Environmental Protection Agency (EPA). The procedures may involve preliminary sample treatment by acid digestion, using either hotplate or microwave oven, or filtration (EPA Method 3005A). Instrumental analysis is by atomic absorption/emission spectrophotometry (EPA Method 7000 series), inductively coupled plasma - optical emission spectrophotometry (EPA Method 6010B), and/or inductively coupled plasma - mass spectrometry (EPA Method 6020).

Recommended Holding Time:
  Sample: 6 months
  Reference: EPA

Laboratory Location: ALS Environmental, Vancouver

Results contained within this certificate relate only to the samples as submitted.

This Certificate Of Analysis shall only be reproduced in full, except with the written approval of ALS Environmental.

End of Report

ALS Environmental

CERTIFICATE OF ANALYSIS

Date:	December 15, 2005
ALS File No.	W8046
Report On:	Soil & Water Analysis
Report To:	**Royal Roads University** Applied Research Division 2005 Sooke Road Victoria, BC V9B 5Y2
Attention:	**Dr. Matthew Dodd**
Received:	November 26, 2005

ALS ENVIRONMENTAL
per:

Sime Buric, B.Sc. - Client Services
Can Dang, B.Sc. - Project Chemist

File No. W8046

RESULTS OF ANALYSIS - Sediment/Soil

Sample ID			OECD-A	OECD-B	OECD-C	OECD-D
ALS ID			*1*	*2*	*3*	*4*
Physical Tests						
Moisture	%		0.96	0.99	0.96	0.93
Total Metals						
Aluminum	T-Al		27400	14000	23900	26100
Antimony	T-Sb		<40	<40	<40	<40
Arsenic	T-As		<200	<200	<200	<200
Barium	T-Ba		3120	3040	3240	26.0
Beryllium	T-Be		<1.0	<1.0	<1.0	<1.0
Bismuth	T-Bi		<40	<40	<40	<40
Cadmium	T-Cd		<4.0	<4.0	<4.0	<4.0
Calcium	T-Ca		2210	2110	2160	2240
Chromium	T-Cr		14.4	9.7	12.9	14.1
Cobalt	T-Co		<4.0	<4.0	<4.0	<4.0
Copper	T-Cu		5.4	5.7	5.9	5.8
Iron	T-Fe		1150	830	1050	1130
Lead	T-Pb		<100	<100	<100	<100
Lithium	T-Li		4.6	<4.0	4.2	4.8
Magnesium	T-Mg		300	250	290	300
Manganese	T-Mn		14.3	13.1	14.1	14.7
Molybdenum	T-Mo		<8.0	<8.0	<8.0	<8.0
Nickel	T-Ni		<10	<10	<10	<10
Phosphorus	T-P		150	130	140	150
Potassium	T-K		<400	<400	<400	<400
Selenium	T-Se		<100	<100	<100	<100
Silver	T-Ag		<4.0	<4.0	<4.0	<4.0
Strontium	T-Sr		68.6	55.7	66.5	28.5
Thallium	T-Tl		<100	<100	<100	<100
Tin	T-Sn		<20	<20	<20	<20
Titanium	T-Ti		71.9	37.1	65.0	68.1
Vanadium	T-V		7.5	4.7	7.6	7.2
Zinc	T-Zn		5.7	3.9	5.6	6.8
Organic Parameters						
Total Organic Carbon		C	2.84	2.87	2.73	2.84

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

File No. W8046

RESULTS OF ANALYSIS - Water

Sample ID		DIL-1A	DIL-1B	DIL-1C	DIL-2A	DIL-2B
ALS ID		*5*	*6*	*7*	*8*	*9*
Total Metals						
Barium	T-Ba	8.49	7.93	7.80	7.19	7.22

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

RESULTS OF ANALYSIS - Water

Sample ID		DIL-2C	DIL-3A	DIL-3B	DIL-3C	DIL-4A
ALS ID		*10*	*11*	*12*	*13*	*14*
Total Metals						
Barium	T-Ba	7.77	7.61	9.95	10.0	9.95

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

RESULTS OF ANALYSIS - Water

Sample ID		DIL-4B	DIL-4C	DIL-5A	DIL-5B	DIL-5C
ALS ID		*15*	*16*	*17*	*18*	*19*
Total Metals						
Barium	T-Ba	10.0	10.0	10.8	12.2	10.8

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

File No. W8046

RESULTS OF ANALYSIS - Water

Sample ID		BF-1A	BF-1B	BF-1C	BF-2A	BF-2B
ALS ID		*20*	*21*	*22*	*23*	*24*
Total Metals						
Barium	T-Ba	10.1	9.89	10.1	10.2	9.98

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

File No. W8046

RESULTS OF ANALYSIS - Water

Sample ID		BF-2C	BF-3A	BF-3B	BF-3C	BF-4A
ALS ID		*25*	*26*	*27*	*28*	*29*
Total Metals						
Barium	T-Ba	10.1	11.5	10.6	10.4	10.2

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

RESULTS OF ANALYSIS - Water

Sample ID		BF-4B	BF-4C	OECD-AX	OECD-BX	OECD-CX
ALS ID		*30*	*31*	*32*	*33*	*34*
Total Metals						
Barium	T-Ba	10.2	10.0	11.7	11.7	11.6

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

RESULTS OF ANALYSIS - Water

Sample ID		OECD-DX	BL05-12	BL05-13	SR05-12	SR05-13
ALS ID		*35*	*36*	*37*	*38*	*39*
Total Metals						
Barium	T-Ba	0.078	<0.010	0.016	1.41	1.37

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

Appendix 1 - QUALITY CONTROL - Replicates

Sediment/Soil			OECD-A	OECD-A QC # 477399
Physical Tests				
Moisture	%		0.96	0.96
Total Metals				
Aluminum	T-Al		27400	18900
Antimony	T-Sb		<40	<40
Arsenic	T-As		<200	<200
Barium	T-Ba		3120	3180
Beryllium	T-Be		<1.0	<1.0
Bismuth	T-Bi		<40	<40
Cadmium	T-Cd		<4.0	<4.0
Calcium	T-Ca		2210	2080
Chromium	T-Cr		14.4	11.5
Cobalt	T-Co		<4.0	<4.0
Copper	T-Cu		5.4	5.6
Iron	T-Fe		1150	950
Lead	T-Pb		<100	<100
Lithium	T-Li		4.6	<4.0
Magnesium	T-Mg		300	260
Manganese	T-Mn		14.3	13.2
Molybdenum	T-Mo		<8.0	<8.0
Nickel	T-Ni		<10	<10
Phosphorus	T-P		150	130
Potassium	T-K		<400	<400
Selenium	T-Se		<100	<100
Silver	T-Ag		<4.0	<4.0
Strontium	T-Sr		68.6	60.7
Thallium	T-Tl		<100	<100
Tin	T-Sn		<20	<20
Titanium	T-Ti		71.9	50.6
Vanadium	T-V		7.5	5.3
Zinc	T-Zn		5.7	4.6
Organic Parameters				
Total Organic Carbon		C	2.84	2.90

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

Appendix 1 - QUALITY CONTROL - Replicates

Water		DIL-2C	DIL-2C QC # 477400	DIL-5C	DIL-5C QC # 477401
Total Metals					
Barium	T-Ba	7.77	8.30	10.8	10.9

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

File No. W8046

Appendix 1 - QUALITY CONTROL - Replicates

Water		OECD-BX	OECD-BX QC # 477402
Total Metals			
Barium	T-Ba	11.7	11.7

Total Organic Carbon results are expressed as percent, dry weight basis.
< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

Appendix 2 - METHODOLOGY

Outlines of the methodologies utilized for the analysis of the samples submitted are as follows

Moisture in Sediment/Soil

This analysis is carried out gravimetrically by drying the sample at 103 C for a minimum of six hours.

Recommended Holding Time:
Sample: 14 days
Reference: Puget

Laboratory Location: ALS Environmental, Vancouver

Metals in Sediment/Soil

This analysis is carried out using procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846 Method 3050B or Method 3051, published by the United States Environmental Protection Agency (EPA). The sample is manually homogenized and a representative subsample of the wet material is weighed. The sample is then digested by either hotplate or microwave oven using a 1:1 ratio of nitric acid and hydrochloric acid. Instrumental analysis is by atomic absorption/emission/fluorescence spectrophotometry (EPA Method 7000 series), inductively coupled plasma - optical emission spectrophotometry (EPA Method 6010B), and/or inductively coupled plasma - mass spectrometry (EPA Method 6020).

Method Limitation: This method is not a total digestion technique. It is a very strong acid digestion that is intended to dissolve those metals that may become "environmentally available." By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment.

Recommended Holding Time:
Sample/Extract: 6 months (Mercury = 28 days)
Reference: EPA

Laboratory Location: ALS Environmental, Vancouver

Total Carbon, Total Organic Carbon and Inorganic Carbon in

Sediment/Soil
This analysis is carried out in accordance with U.S. EPA Method 9060A (Publ. # SW-846 3rd ed., Washington, DC 20460). Using a Leco analyzer Total Carbon is determined by high temperature oxidation of carbon to carbon dioxide which is then measured by means of a nondispersive infrared analyzer. Inorganic Carbon is determined by means of a CO2 coulometer. A prepared sample is acidified in a heated reaction vessel to evolve inorganic carbon as carbon dioxide. The resulting carbon dioxide is then measured by chemical reaction in the CO2 coulometer. Total Organic Carbon is determined as the difference between Total and Inorganic Carbons.

Recommended Holding Time:
Sample: 14 days
Reference: Puget

Laboratory Location: ALS Chemex, North Vancouver

Metals in Water

This analysis is carried out using procedures adapted from "Standard Methods for the Examination of Water and Wastewater" 20th Edition 1998 published by the American Public Health Association, and with procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846 published by the United States Environmental Protection Agency (EPA). The procedures may involve preliminary sample treatment by acid digestion, using either hotplate or microwave oven, or filtration (EPA Method 3005A). Instrumental analysis is by atomic absorption/emission spectrophotometry (EPA Method 7000 series), inductively coupled plasma - optical emission spectrophotometry (EPA Method 6010B), and/or inductively coupled plasma - mass spectrometry (EPA Method 6020).

Recommended Holding Time:
Sample: 6 months
Reference: EPA

Laboratory Location: ALS Environmental, Vancouver

Results contained within this certificate relate only to the samples as submitted.

This Certificate Of Analysis shall only be reproduced in full, except with the written approval of ALS Environmental.

End of Report

ALS Environmental

CERTIFICATE OF ANALYSIS

Date: October 11, 2006

ALS File No. Z3003

Report On: PBET Extract/Soil Analysis

Report To: **Royal Roads University**
School of Environment & Sustain.
2005 Sooke Road
Victoria, BC
V9B 5Y2

Attention: **Dr. Matthew Dodd**

Received: September 25, 2006

ALS ENVIRONMENTAL
per:

Heather A. Ross-Easton, B.Sc. - Account Manager
Can Dang, B.Sc. - Senior Account Manager

ALS CANADA LTD.
1988 Triumph Street, Vancouver, BC Canada V5L 1K5
Phone: 604-253-4188 Fax: 604-253-6700 Website: www.alsenviro.com

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File No. Z3003

RESULTS OF ANALYSIS - Extract[1]

Sample ID		CaC1-2A	CaC1-2B	CaC1-3A	CaC1-3B	CaC14A
ALS ID		*1*	*2*	*3*	*4*	*5*
Total Metals						
Barium	T-Ba	3.11	1.30	1.83	1.92	1.22

< = Less than the detection limit indicated.

[1] Results are expressed as milligrams per litre except where noted.

RESULTS OF ANALYSIS - Extract[1]

Sample ID		CaC1-4B	CaC1-5A	CaC1-5B	BL06-016	S2-D1A
ALS ID		*6*	*7*	*8*	*9*	*10*
Total Metals						
Barium	T-Ba	1.27	1.19	1.26	0.130	9.70

< = Less than the detection limit indicated.

[1] Results are expressed as milligrams per litre except where noted.

RESULTS OF ANALYSIS - Extract[1]

Sample ID		S2-D1B	S2-D1C	S2-D2A	S2-D2B	S2-D2C
ALS ID		*11*	*12*	*13*	*14*	*15*
Total Metals						
Barium	T-Ba	9.79	10.1	11.3	11.4	11.4

< = Less than the detection limit indicated.
[1] Results are expressed as milligrams per litre except where noted.

File No. Z3003

RESULTS OF ANALYSIS - Extract[1]

Sample ID		S2-D3A	S2-D3B	S2-D3C	S3-D1A	S3-D1B
ALS ID		*16*	*17*	*18*	*19*	*20*
Total Metals						
Barium	T-Ba	3.27	3.27	3.24	11.9	11.9

< = Less than the detection limit indicated.

[1] Results are expressed as milligrams per litre except where noted.

RESULTS OF ANALYSIS - Extract[1]

Sample ID		S3-D1C	S3-D2A	S3-D2B	S3-D2C	S3-D3A
ALS ID		*21*	*22*	*23*	*24*	*25*
Total Metals						
Barium	T-Ba	11.5	6.71	6.42	6.77	0.788

< = Less than the detection limit indicated.

[1] Results are expressed as milligrams per litre except where noted.

File No. Z3003

RESULTS OF ANALYSIS - Extract[1]

Sample ID		S3-D3C	S3-D3B	S4-D1A	S4-D1B	S4-D1C
ALS ID		*26*	*27*	*28*	*29*	*30*
Total Metals						
Barium	T-Ba	0.942	0.524	1.80	1.85	1.83

< = Less than the detection limit indicated.

[1] Results are expressed as milligrams per litre except where noted.

File No. Z3003

RESULTS OF ANALYSIS - Extract[1]

Sample ID		S4-D2A	S4-D2B	S4-D2C	S4-D3A	S4-D3B
ALS ID		*31*	*32*	*33*	*34*	*35*
Total Metals						
Barium	T-Ba	0.197	0.192	0.204	<0.050	<0.050

< = Less than the detection limit indicated.

[1] Results are expressed as milligrams per litre except where noted.

File No. Z3003

RESULTS OF ANALYSIS - Extract[1]

Sample ID		S4-D3C	S5-D1A	S5-D1B	S5-D1C	S5-D2A
ALS ID		*36*	*37*	*38*	*39*	*40*
Total Metals						
Barium	T-Ba	<0.050	7.58	8.07	8.02	2.91

< = Less than the detection limit indicated.

[1] Results are expressed as milligrams per litre except where noted.

File No. Z3003

RESULTS OF ANALYSIS - Extract[1]

Sample ID		S5-D2B	S5-D2C	S5-D3A	S5-D3B	S5-D3C
ALS ID		*41*	*42*	*43*	*44*	*45*
Total Metals						
Barium	T-Ba	3.11	3.40	0.383	0.429	0.425

< = Less than the detection limit indicated.

[1] Results are expressed as milligrams per litre except where noted.

File No. Z3003

RESULTS OF ANALYSIS - Sediment/Soil[1]

Sample ID		ST2-F4A	ST2-F4B	ST2-F4C	ST5-F4A	ST5-F4B
ALS ID		*46*	*47*	*48*	*49*	*50*
Physical Tests						
Moisture	%	0.96	1.01	1.07	2.84	2.81
Total Metals						
Barium	T-Ba	876	1120	1190	1290	1440

< = Less than the detection limit indicated.

[1] Results are expressed as milligrams per dry kilogram except where noted.

File No. Z3003

RESULTS OF ANALYSIS - Sediment/Soil[1]

Sample ID		ST5-F4C
ALS ID		*51*
Physical Tests		
Moisture	%	3.04
Total Metals		
Barium	T-Ba	1600

< = Less than the detection limit indicated.
[1]Results are expressed as milligrams per dry kilogram except where noted.

Appendix 1 - QUALITY CONTROL - Replicates

Extract[1]		S3-D1C	S3-D1C	S4-D1A	S4-D1A
			QC # 524020		QC # 524021
Total Metals					
Barium	T-Ba	11.5	11.5	1.80	1.86

< = Less than the detection limit indicated.
Results are expressed as milligrams per litre except where noted.

File No. Z3003

Appendix 1 - QUALITY CONTROL - Replicates

Extract[1]		S5-D1A	S5-D1A QC # 524022
Total Metals			
Barium	T-Ba	7.58	7.60

< = Less than the detection limit indicated.
Results are expressed as milligrams per litre except where noted.

Appendix 1 - QUALITY CONTROL - Replicates

Sediment/Soil[1]		ST2-F4B	ST2-F4B QC # 524023	ST5-F4C	ST5-F4C QC # 524024
Physical Tests					
Moisture	%	1.01	0.80	3.04	2.94
Total Metals					
Barium	T-Ba	1120	1250	1600	1230

< = Less than the detection limit indicated.
Results are expressed as milligrams per dry kilogram except where noted.

File No. Z3003

Appendix 1 - QUALITY CONTROL - Reference Materials

Sediment/Soil

		Result	Target	Units	DL	ALSQC#	Method
CANMET Till Reference Material, TILL-1							
Total Metals							
Barium	T-Ba	86.6	**702**	mg/kg	1	524558	a
Barium	T-Ba	87.8	**702**	mg/kg	1	524562	a

Methods:
a = CSR/HCl-HNO3/ICPOES

File No. Z3003

Appendix 1 - QUALITY CONTROL - Blanks

Sediment/Soil

		Result	Target	Units	DL	ALSQC#	Method
Total Metals							
Barium	T-Ba	<1.0	<1.0	mg/kg	1	524555	a
Barium	T-Ba	<1.0	<1.0	mg/kg	1	524556	a
Barium	T-Ba	<1.0	<1.0	mg/kg	1	524559	a
Barium	T-Ba	<1.0	<1.0	mg/kg	1	524560	a
Barium	T-Ba	<1.0	<1.0	mg/kg	1	526440	b
Barium	T-Ba	<1.0	<1.0	mg/kg	1	526441	b

Methods:
a = CSR/HCl-HNO3/ICPOES
b = HCl-HNO3/ICPOES
Bold Targets indicate uncertified targets

Appendix 2 - METHODOLOGY

Outlines of the methodologies utilized for the analysis of the samples submitted are as follows

Metals in Water

This analysis is carried out using procedures adapted from "Standard Methods for the Examination of Water and Wastewater" 21th Edition 2005 published by the American Public Health Association, and with procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846 published by the United States Environmental Protection Agency (EPA). The procedures may involve preliminary sample treatment by acid digestion, using either hotplate or microwave oven, or filtration (EPA Method 3005A). Instrumental analysis is by atomic absorption/emission spectrophotometry (EPA Method 7000 series), inductively coupled plasma - optical emission spectrophotometry (EPA Method 6010B), and/or inductively coupled plasma - mass spectrometry (EPA Method 6020).

Recommended Holding Time:
Sample: 6 months
Reference: EPA

Laboratory Location: ALS Environmental, Vancouver

Moisture in Sediment/Soil

This analysis is adapted from ASTM method D2794-00 "Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils". A sub-sample is dried at 105 Celsius for a minimum of six hours.

Recommended Holding Time:
Sample: not specified for refrigerated samples
Reference: Puget, BCELM

Laboratory Location: ALS Environmental, Vancouver

Metals in Sediment/Soil

This analysis is carried out using procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846 Method 3050B or Method 3051, published by the United States Environmental Protection Agency (EPA). The sample is manually homogenized and a representative subsample of the wet material is weighed. The sample is then digested by either hotplate or microwave oven using a 1:1 ratio of nitric acid and hydrochloric acid. Instrumental analysis is by atomic absorption/emission/fluorescence spectrophotometry (EPA Method 7000 series), inductively coupled plasma - optical emission spectrophotometry (EPA Method 6010B), and/or inductively coupled plasma - mass spectrometry (EPA Method 6020).

Method Limitation: This method is not a total digestion technique. It is a very strong acid digestion that is intended to dissolve those metals that may become "environmentally available." By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment.

Recommended Holding Time:
Sample/Extract: 6 months (Mercury = 28 days)
Reference: EPA

Laboratory Location: ALS Environmental, Vancouver

Results contained within this certificate relate only to the samples as submitted.

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End of Report

ALS Environmental

CERTIFICATE OF ANALYSIS

Date:	June 12, 2006
ALS File No.	X6353
Report On:	PBET Soil Analysis
Report To:	**Royal Roads University** Applied Research Division 2005 Sooke Road Victoria, BC V9B 5Y2
Attention:	**Dr. Matthew Dodd**
Received:	May 26, 2006

ALS ENVIRONMENTAL
per:

Heather A. Ross-Easton, B.Sc. - Account Manager
Can Dang, B.Sc. - Senior Account Manager

ALS CANADA LTD
1988 Triumph Street, Vancouver, BC Canada V5L 1K5
Phone: 604-253-4188 Fax: 604-253-6700 Website: www.alsenviro.com

A Campbell Brothers Limited Company

File No. X6353

RESULTS OF ANALYSIS - Sediment/Soil

Sample ID			ST4-F1A	ST4-F1B	ST4-F1C	ST4-F2A	ST4-F2B
ALS ID			*1*	*2*	*3*	*4*	*5*
Physical Tests							
pH			7.71	7.73	7.80	7.65	7.77
Total Metals							
Antimony	T-Sb		<10	<10	<10	<10	<10
Arsenic	T-As		6.6	7.1	6.8	7.0	7.6
Barium	T-Ba		1720	1550	1300	1100	1100
Beryllium	T-Be		0.72	0.75	0.73	0.75	0.75
Cadmium	T-Cd		<0.50	<0.50	<0.50	<0.50	<0.50
Chromium	T-Cr		20.7	21.0	20.9	21.9	21.2
Cobalt	T-Co		9.2	9.3	9.1	9.4	9.5
Copper	T-Cu		32.2	32.7	33.7	39.7	42.4
Lead	T-Pb		<50	<50	<50	<50	<50
Mercury	T-Hg		0.096	<0.050	0.063	0.076	0.081
Molybdenum	T-Mo		<4.0	<4.0	<4.0	<4.0	<4.0
Nickel	T-Ni		27.0	27.8	26.8	28.3	28.5
Selenium	T-Se		<2.0	<2.0	<2.0	<2.0	<2.0
Silver	T-Ag		<2.0	<2.0	<2.0	<2.0	<2.0
Tin	T-Sn		<5.0	<5.0	<5.0	<5.0	<5.0
Vanadium	T-V		34.0	30.4	33.7	35.1	33.1
Zinc	T-Zn		86.0	86.4	84.0	90.7	90.7
Organic Parameters							
Total Organic Carbon		C	1.84	1.90	1.92	2.08	2.10

Results are expressed as milligrams per dry kilogram except where noted.
< = Less than the detection limit indicated.
Total Organic Carbon results are expressed as percent, dry weight basis.

RESULTS OF ANALYSIS - Sediment/Soil

Sample ID			ST4-F2C	ST4-F3A	ST4-F3B	ST4-F3C	ST4-F4A
ALS ID			*6*	*7*	*8*	*9*	*10*
Physical Tests							
pH			7.74	7.72	7.76	7.74	8.07
Total Metals							
Antimony	T-Sb		<10	<10	<10	<10	<10
Arsenic	T-As		6.1	6.0	6.6	6.6	6.5
Barium	T-Ba		1190	1210	1340	1230	1150
Beryllium	T-Be		0.78	0.79	0.84	0.83	0.84
Cadmium	T-Cd		<0.50	<0.50	<0.50	<0.50	<0.50
Chromium	T-Cr		22.1	22.5	23.4	23.6	24.8
Cobalt	T-Co		9.3	9.4	9.5	9.5	9.7
Copper	T-Cu		41.4	55.2	54.8	56.3	53.9
Lead	T-Pb		<50	<50	<50	<50	<50
Mercury	T-Hg		0.077	0.081	0.078	0.091	0.095
Molybdenum	T-Mo		<4.0	<4.0	<4.0	<4.0	<4.0
Nickel	T-Ni		28.8	28.6	28.9	29.1	30.3
Selenium	T-Se		<2.0	<2.0	<2.0	<2.0	<2.0
Silver	T-Ag		<2.0	<2.0	<2.0	<2.0	<2.0
Tin	T-Sn		<5.0	5.5	5.9	5.1	<5.0
Vanadium	T-V		33.3	34.0	37.8	35.3	38.9
Zinc	T-Zn		92.8	95.3	95.9	96.0	102
Organic Parameters							
Total Organic Carbon		C	2.10	2.07	2.10	2.06	2.06

Results are expressed as milligrams per dry kilogram except where noted.
< = Less than the detection limit indicated.
Total Organic Carbon results are expressed as percent, dry weight basis.

File No. X6353

RESULTS OF ANALYSIS - Sediment/Soil

Sample ID			ST4-F4B	ST4-F4C	ST4C-F1A	ST4C-F1B	ST4C-F1C
ALS ID			*11*	*12*	*13*	*14*	*15*
Physical Tests							
pH			7.69	7.72	7.93	7.97	7.90
Total Metals							
Antimony	T-Sb		<10	<10	<10	<10	<10
Arsenic	T-As		7.1	7.2	5.6	5.5	5.5
Barium	T-Ba		1040	1190	235	239	238
Beryllium	T-Be		0.82	0.81	0.77	0.78	0.79
Cadmium	T-Cd		<0.50	<0.50	<0.50	<0.50	<0.50
Chromium	T-Cr		23.3	22.5	18.4	19.1	18.7
Cobalt	T-Co		9.7	9.6	7.8	7.9	7.8
Copper	T-Cu		53.9	51.7	31.0	31.2	31.7
Lead	T-Pb		<50	<50	<50	<50	<50
Mercury	T-Hg		0.081	0.091	0.063	0.063	0.063
Molybdenum	T-Mo		<4.0	<4.0	<4.0	<4.0	<4.0
Nickel	T-Ni		30.6	30.0	24.6	25.2	24.6
Selenium	T-Se		<2.0	<2.0	<2.0	<2.0	<2.0
Silver	T-Ag		<2.0	<2.0	<2.0	<2.0	<2.0
Tin	T-Sn		<5.0	<5.0	<5.0	<5.0	<5.0
Vanadium	T-V		35.2	34.9	29.5	32.4	32.7
Zinc	T-Zn		103	101	70.1	75.8	71.5
Organic Parameters							
Total Organic Carbon		C	2.10	2.09	0.610	0.670	0.670

Results are expressed as milligrams per dry kilogram except where noted.
< = Less than the detection limit indicated.
Total Organic Carbon results are expressed as percent, dry weight basis.

RESULTS OF ANALYSIS - Extract

Sample ID		BL05-21E	SR05-21E	ST4-F1AE	ST4-F1BE	ST4-F1CE
ALS ID		*16*	*17*	*18*	*19*	*20*
Total Metals						
Barium	T-Ba	<0.020	0.161	4.11	4.23	4.34

Results are expressed as milligrams per dry kilogram except where noted.
< = Less than the detection limit indicated.
Total Organic Carbon results are expressed as percent, dry weight basis.

RESULTS OF ANALYSIS - Extract

Sample ID		ST4-F2AE	ST4-F2BE	ST4-F2CE	ST4-F3AE	ST4-F3BE
ALS ID		*21*	*22*	*23*	*24*	*25*
Total Metals						
Barium	T-Ba	4.94	5.37	5.18	5.59	5.79

Results are expressed as milligrams per dry kilogram except where noted.
< = Less than the detection limit indicated.
Total Organic Carbon results are expressed as percent, dry weight basis.

RESULTS OF ANALYSIS - Extract

Sample ID		ST4-F3CE	ST4-F4AE	ST4-F4BE	ST4-F4CE	ST4C-F1AE
ALS ID		*26*	*27*	*28*	*29*	*30*
Total Metals						
Barium	T-Ba	5.52	5.87	5.66	6.07	0.186

Results are expressed as milligrams per dry kilogram except where noted.
< = Less than the detection limit indicated.
Total Organic Carbon results are expressed as percent, dry weight basis.

File No. X6353

RESULTS OF ANALYSIS - Extract

Sample ID		ST4C-F1BE	ST4C-F1CE
ALS ID		*31*	*32*
Total Metals			
Barium	T-Ba	0.191	0.193

Results are expressed as milligrams per dry kilogram except where noted.
< = Less than the detection limit indicated.
Total Organic Carbon results are expressed as percent, dry weight basis.

Appendix 1 - QUALITY CONTROL - Replicates

Sediment/Soil		ST4-F2A	ST4-F2A QC # 503947	ST4-F4A	ST4-F4A QC # 503948
Physical Tests					
pH		7.65	7.70	8.07	8.03
Total Metals					
Antimony	T-Sb	<10	<10	<10	<10
Arsenic	T-As	7.0	5.7	6.5	6.7
Barium	T-Ba	1100	978	1150	994
Beryllium	T-Be	0.75	0.73	0.84	0.84
Cadmium	T-Cd	<0.50	<0.50	<0.50	<0.50
Chromium	T-Cr	21.9	20.1	24.8	24.6
Cobalt	T-Co	9.4	8.9	9.7	9.7
Copper	T-Cu	39.7	40.0	53.9	52.2
Lead	T-Pb	<50	<50	<50	<50
Mercury	T-Hg	0.076	0.072	0.095	0.092
Molybdenum	T-Mo	<4.0	<4.0	<4.0	<4.0
Nickel	T-Ni	28.3	26.9	30.3	30.5
Selenium	T-Se	<2.0	<2.0	<2.0	<2.0
Silver	T-Ag	<2.0	<2.0	<2.0	<2.0
Tin	T-Sn	<5.0	<5.0	<5.0	<5.0
Vanadium	T-V	35.1	31.0	38.9	37.2
Zinc	T-Zn	90.7	87.6	102	101

Results are expressed as milligrams per dry kilogram except where noted.
< = Less than the detection limit indicated.
Total Organic Carbon results are expressed as percent, dry weight basis.

File No. X6353

Appendix 1 - QUALITY CONTROL - Replicates

Extract		ST4-F4CE	ST4-F4CE QC # 503949
Total Metals			
Barium	T-Ba	6.07	5.85

Results are expressed as milligrams per dry kilogram except where noted.
< = Less than the detection limit indicated.
Total Organic Carbon results are expressed as percent, dry weight basis.

File No. X6353

Appendix 1 - QUALITY CONTROL - Reference Materials

Sediment/Soil

NRC Esquimalt Harbour Sediment, PACS-2

Total Metals

		Result	Target	Units	DL	ALSQC#	Method
Antimony	T-Sb	<10	11	mg/kg	10	504201	a
Arsenic	T-As	26.1	26.2	mg/kg	5	504201	a
Beryllium	T-Be	<0.50	1.00	mg/kg	0.5	504201	a
Cadmium	T-Cd	2.16	2.11	mg/kg	0.5	504201	a
Chromium	T-Cr	49.0	90.7	mg/kg	2	504201	a
Cobalt	T-Co	8.3	11.5	mg/kg	2	504201	a
Copper	T-Cu	324	310	mg/kg	1	504201	a
Lead	T-Pb	170	183	mg/kg	50	504201	a
Mercury	T-Hg	3.08	3.04	mg/kg	0.05	504257	b
Mercury	T-Hg	2.91	3.04	mg/kg	0.05	504479	b
Molybdenum	T-Mo	5.4	5.4	mg/kg	4	504201	a
Nickel	T-Ni	31.6	39.5	mg/kg	5	504201	a
Selenium	T-Se	<2.0	<2.0	mg/kg	2	504201	a
Silver	T-Ag	<2.0	<2.0	mg/kg	2	504201	a
Tin	T-Sn	19.4	19.8	mg/kg	5	504201	a
Vanadium	T-V	78.8	133	mg/kg	2	504201	a
Zinc	T-Zn	342	364	mg/kg	1	504201	a

CANMET Till Reference Material, TILL-1

Total Metals

		Result	Target	Units	DL	ALSQC#	Method
Antimony	T-Sb	<10	**<10**	mg/kg	10	504200	a
Arsenic	T-As	17.2	**18.0**	mg/kg	5	504200	a
Barium	T-Ba	88.8	**702**	mg/kg	1	504200	a
Beryllium	T-Be	<0.50	**2.40**	mg/kg	0.5	504200	a
Cadmium	T-Cd	<0.50	**<0.50**	mg/kg	0.5	504200	a
Chromium	T-Cr	29.5	**65.0**	mg/kg	2	504200	a
Cobalt	T-Co	13.0	**18.0**	mg/kg	2	504200	a
Copper	T-Cu	49.7	**47.0**	mg/kg	1	504200	a
Lead	T-Pb	<50	**<50**	mg/kg	50	504200	a
Mercury	T-Hg	0.112	**0.095**	mg/kg	0.05	504258	b
Mercury	T-Hg	0.101	**0.095**	mg/kg	0.05	504480	b
Molybdenum	T-Mo	<4.0	**<4.0**	mg/kg	4	504200	a
Nickel	T-Ni	17.6	**24.0**	mg/kg	5	504200	a
Selenium	T-Se	<2.0	**2.0**	mg/kg	2	504200	a

Methods:
a = CSR/HCl-HNO3/ICPOES
b = CSR/HCl-HNO3/CVAFS

Appendix 1 - QUALITY CONTROL - Reference Materials

Sediment/Soil

CANMET Till Reference Material, TILL-1

		Result	Target	Units	DL	ALSQC#	Method
Total Metals							
Silver	T-Ag	<2.0	**<2.0**	mg/kg	2	504200	a
Tin	T-Sn	<5.0	**5.0**	mg/kg	5	504200	a
Vanadium	T-V	62.2	**99.0**	mg/kg	2	504200	a
Zinc	T-Zn	73.1	**98.0**	mg/kg	1	504200	a

Methods:
a = CSR/HCl-HNO3/ICPOES

File No. X6353

Appendix 1 - QUALITY CONTROL - Blanks

Sediment/Soil

		Result	Target	Units	DL	ALSQC#	Method
Total Metals							
Antimony	T-Sb	<10	<10	mg/kg	10	504198	a
Arsenic	T-As	<5.0	<5.0	mg/kg	5	504198	a
Barium	T-Ba	<1.0	<1.0	mg/kg	1	504198	a
Beryllium	T-Be	<0.50	<0.50	mg/kg	0.5	504198	a
Cadmium	T-Cd	<0.50	<0.50	mg/kg	0.5	504198	a
Chromium	T-Cr	<2.0	<2.0	mg/kg	2	504198	a
Cobalt	T-Co	<2.0	<2.0	mg/kg	2	504198	a
Copper	T-Cu	<1.0	<1.0	mg/kg	1	504198	a
Lead	T-Pb	<50	<50	mg/kg	50	504198	a
Mercury	T-Hg	<0.050	<0.050	mg/kg	0.05	504255	b
Mercury	T-Hg	<0.050	<0.050	mg/kg	0.05	504256	b
Mercury	T-Hg	<0.050	<0.050	mg/kg	0.05	504477	b
Mercury	T-Hg	<0.050	<0.050	mg/kg	0.05	504478	b
Molybdenum	T-Mo	<4.0	<4.0	mg/kg	4	504198	a
Nickel	T-Ni	<5.0	<5.0	mg/kg	5	504198	a
Selenium	T-Se	<2.0	<2.0	mg/kg	2	504198	a
Silver	T-Ag	<2.0	<2.0	mg/kg	2	504198	a
Tin	T-Sn	<5.0	<5.0	mg/kg	5	504198	a
Vanadium	T-V	<2.0	<2.0	mg/kg	2	504198	a
Zinc	T-Zn	4.8	<1.0	mg/kg	1	504198	a

Methods:
a = CSR/HCl-HNO3/ICPOES
b = CSR/HCl-HNO3/CVAFS
Bold Targets indicate uncertified targets

Appendix 2 - METHODOLOGY

Outlines of the methodologies utilized for the analysis of the samples submitted are as follows

pH in Soil

This analysis is carried out in accordance with procedures described in "Soil Sampling and Methods of Analysis" (CSSS). The procedure involves mixing the air-dried sample with deionized/distilled water. The pH of the solution is then measured using a standard pH probe. A one to two ratio of sediment to water is used for mineral soils and a one to ten ratio is used for highly organic soils.

Laboratory Location: ALS Environmental, Vancouver

Metals in Sediment/Soil

This analysis is carried out using procedures from CSR Analytical Method 8 "Strong Acid Leachable Metals (SALM) in Soil", BC Ministry of Environment, Lands and Parks, 26 June 2001, and procedures adapted from "Test Methods for Evaluating Solid Waste", SW-846 Method 3050B or Method 3051, United States Environmental Protection Agency (EPA). The sample is manually homogenized, dried at 60 degrees Celsius, sieved through a 2 mm (10 mesh) sieve, and a representative subsample of the dry material is weighed. The sample is then digested at 90 degrees Celsius for 2 hours by either hotplate or block digester using a 1:1 ratio of concentrated nitric and hydrochloric acids. Instrumental analysis is by atomic absorption/fluorescence spectrophotometry (EPA Method 7000 series), inductively coupled plasma - optical emission spectrophotometry (EPA Method 6010B), and/or inductively coupled plasma - mass spectrometry (EPA Method 6020).

Method Limitation: This method is not a total digestion technique. It is a very strong acid digestion that is intended to dissolve those metals that may be environmentally available. By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment.

Recommended Holding Time:
    Sample: 6 months (Hg = 28 days)
    Extract: 6 months (Hg = 28 days, Sb & Sn = 7 days)
    Reference: BCMELP

Laboratory Location: ALS Environmental, Vancouver

Total Carbon, Total Organic Carbon and Inorganic Carbon in

Sediment/Soil

This analysis is carried out in accordance with U.S. EPA Method 9060A (Publ. # SW-846 3rd ed., Washington, DC 20460). Using a Leco analyzer Total Carbon is determined by high temperature oxidation of carbon to carbon dioxide which is then measured by means of a nondispersive infrared analyzer. Inorganic Carbon is determined by means of a CO2 coulometer. A prepared sample is acidified in a heated reaction vessel to evolve inorganic carbon as carbon dioxide. The resulting carbon dioxide is then measured by chemical reaction in the CO2 coulometer. Total Organic Carbon is determined as the difference between Total and Inorganic Carbons.

Recommended Holding Time:
Sample: 14 days
Reference: Puget

Laboratory Location: ALS Chemex, North Vancouver

Metals in Water

This analysis is carried out using procedures adapted from "Standard Methods for the Examination of Water and Wastewater" 20th Edition 1998 published by the American Public Health Association, and with procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846 published by the United States Environmental Protection Agency (EPA). The procedures may involve preliminary sample treatment by acid digestion, using either hotplate or microwave oven, or filtration (EPA Method 3005A). Instrumental analysis is by atomic absorption/emission spectrophotometry (EPA Method 7000 series), inductively coupled plasma - optical emission spectrophotometry (EPA Method 6010B), and/or inductively coupled plasma - mass spectrometry (EPA Method 6020).

Recommended Holding Time:
Sample: 6 months
Reference: EPA

Laboratory Location: ALS Environmental, Vancouver

Results contained within this certificate relate only to the samples as submitted.

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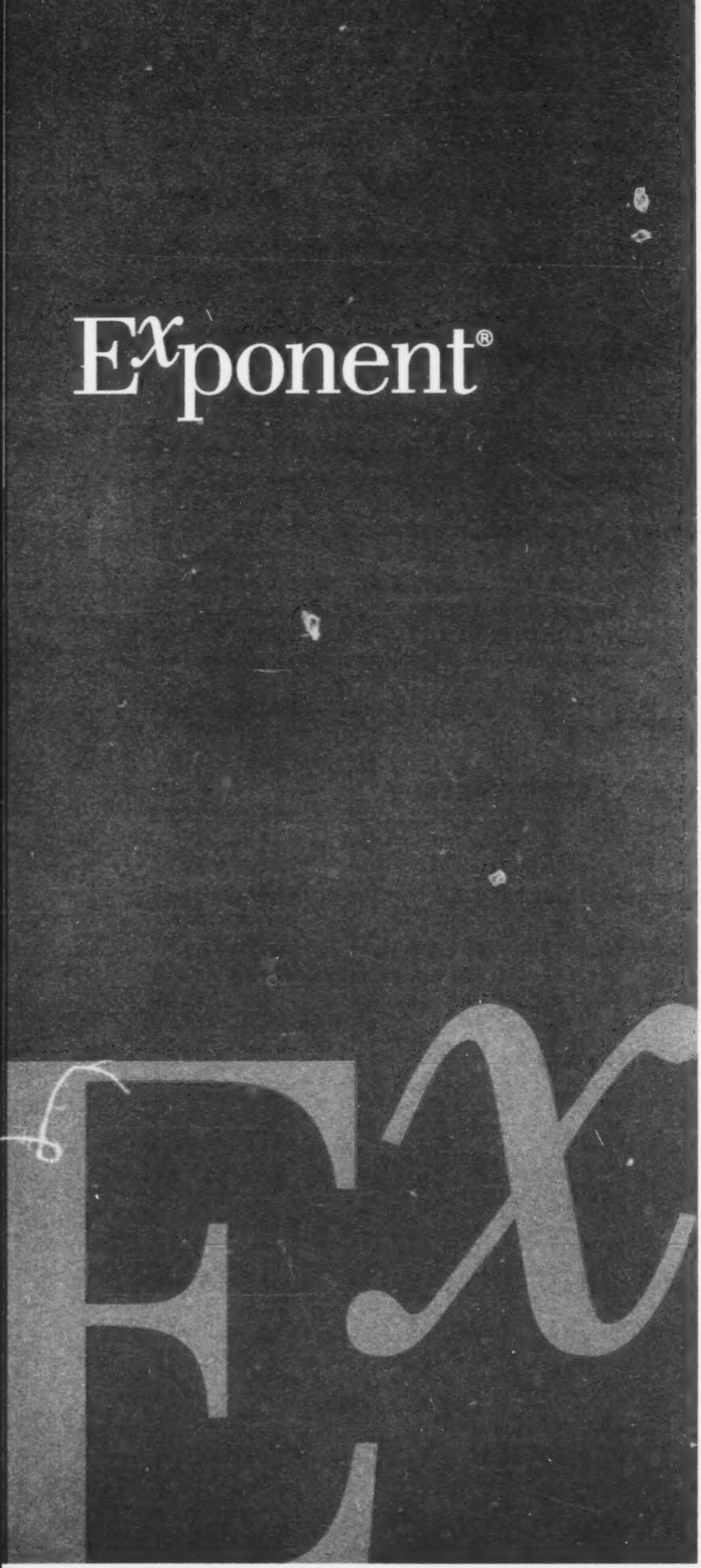
End of Report

APPENDIX B

EXPONENT BIOACCESSIBILITY STUDY



Bioaccessibility of Barium from Barite-Containing Canada Soils

Prepared for
Canadian Association of Petroleum
Producers

Exponent

Bioaccessibility of Barium from Barite-Containing Canada Soils

Prepared for

Canadian Association of Petroleum Producers

Prepared by

Exponent
4875 Pearl East Circle, Suite 201
Boulder, Colorado 80301

February 2007

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Appendix B. Exponent Memo, June 28, 2006: Information on Early Stages of Soil Extraction Testing

Background

At the request of the Canadian Association of Petroleum Producers (CAPP), Exponent has conducted extraction testing of barium from barite-containing soils. The goal of the research has been to understand whether chemical-, site-, or soil-specific factors are controlling the dissolution of barium from the soils. Regulatory toxicity criteria have been developed to be protective of human and ecological exposures to barium (e.g., reference dose, or RfD); these criteria are based on exposure to soluble forms of barium. If barium is not soluble from the barite ($BaSO_4$) phases in barite-containing soils, then it may not be appropriate to evaluate potential human or ecological effects based on total barium concentrations in the soils. Instead, an adjustment to account for the relative oral bioavailability (RBA) (i.e., the amount of barium absorbed from the soil relative to the amount of barium absorbed following exposure to soluble barium) may be required to better understand exposures from the soils of concern.

Bioavailability Research

Research conducted over the last two decades has established that the bioavailability of metals and other contaminants in soil can be significantly lower than the bioavailability of soluble forms (NRC 2003; SERDP 2005; Casteel et al. 1997; Freeman et al. 1993 and 1995; Oomen 2000; Rodriquez et al. 2003; Roberts et al. 2002, 2007). In addition to conducting studies using animals to understand this RBA of chemicals from soils, *in vitro*, or bench top, methods have also been developed that are useful in evaluating the RBA of chemicals from soil (Drexler and Brattin 2007, Oomen 2000, Ruby 1999). Of these, one *in vitro* method, the "Relative Bioavailability Leaching Procedure" (RBALP; Drexler and Brattin 2007; Ruby et al. 1999) has been validated as a method that is predictive of the RBA of lead as measured in an animal model. Specifically, 19 soils from 10 different sites have been evaluated for relative oral bioavailability of lead *in vivo* using the immature swine research model (Casteel et al. 2006). Corresponding data have been generated for *in vitro* extraction of lead from the same soils, and the results from the *in vitro* method were found to correspond well with the *in vivo* results, with good reproducibility across the laboratories conducting the *in vitro* extractions (Drexler and Brattin 2007). This evaluation of lead serves as the basis for upcoming guidance from the U.S. Environmental Protection Agency (USEPA), recommending use of the RBALP *in vitro* method to assess the RBA of lead from soils on a site-specific basis.

This extensive research on lead has shown that the solubility and oral bioavailability of lead from soils is controlled by several site-specific factors, including particle size, the specific form (mineralogy) of lead in the soil, and other physical characteristics of the soil particles (Ruby 1999). Geochemical considerations suggest that barium would be expected to behave similarly to lead with regard to solubility, or could even demonstrate lower levels of solubility in some chemical forms. For example, research conducted on the dissolution of sulfate chemicals, including lead sulfate (anglesite, $PbSO_4$) and barium sulfate (barite, $BaSO_4$), indicate that the dissolution of barite is slower than the solubility of anglesite within the pH range of 2 to 10 (Dove and Czank 1995). Based on this information, an initial premise of the evaluation of barite-containing soils was that the *in vitro* method developed for lead might be an appropriate tool to provide insights into understanding the relative oral bioavailability of barite from soils. Therefore, a course of extraction testing was implemented for the barite-containing soils, using the RBALP as the basis for the *in vitro* extraction process.

Extraction Methods

All soil extractions were conducted using the *in vitro*, physiologically-based extraction test (PBET) method developed in the laboratory of Dr. John Drexler at the University of Colorado in Boulder. This method has been published by Ruby et al. (1993), and more recently by Drexler and Brattin (2007, accepted). This extraction method utilizes a glycine buffer system, at pH 1.5, and employs end-over-end rotation of samples in extraction fluid, at 37 °C, for one hour. Following extraction, the samples were filtered, and the extraction fluid was evaluated for barium concentrations by ICP/MS. The bioaccessible fraction was determined by dividing the mass of barium extracted during this process to the mass of barium originally in the solid phase. For barium-containing soils (Phase III), bioaccessibility data are reported based on soil barium concentrations determined by USEPA method 3050B, and separately, based on soil barium concentrations determined by energy dispersive x-ray fluorescence (XRF). Standard operating procedures for the extraction process are provided in Appendix A.

Modifications were incorporated into the research design to address additional questions and provide data to understand factors that may be influencing the results of the extraction testing. Specifically, modifications were incorporated into the extraction testing scheme to assess the effect of particle size, the soil:fluid ratio, and the pH for the extraction testing.

Effect of Particle Size on the Dissolution of Barium

The RBALP method specifies that extraction testing be conducted on the <250-μm particle size fraction because this is the size fraction assumed by USEPA to adhere to the skin and, thus, to be relevant for inadvertent ingestion exposures from hand-to-mouth contact (USEPA 2000; Casteel et al. 2006). As mentioned above and discussed extensively in Ruby et al. (1999), soil particle size is one of the factors controlling dissolution. Therefore, the evaluation of barite soils was conducted on three specific particle size fractions:

- Less than 2-mm soil particle size, because this particle size is frequently the basis for chemical contamination/soil characterization studies.

- Less than 250-μm particle size fraction, per the RBALP method specifications.

- Less than 38-μm particle size fraction. (Staff within Health Canada have a perspective different from that of USEPA regarding the relevant particle size fraction for inadvertent soil ingestion exposures from hand-to-mouth contact, and have indicated that the relevant size fraction may be as small as <10 μm. Sieving to this extremely small particle size introduces practical considerations, such as electrostatic adherence of the particles to the sieve, and ensuring adequate soil mass for extraction testing. Therefore, the compromise of evaluating the <38-μm particle size fraction was implemented.)

Effect of the Solid:Fluid Ratio on Dissolution of Barium

In addition to the specifications with regard to particle size, the RBALP method also specifies that the extractions be conducted using a solid:fluid ratio of 1:100. More specifically, the method subjects one gram of solid to extraction into 100 mL of extraction fluid. Because dissolution kinetics are driven in part by this relation, CAPP requested that initial phases of the research undertaken by Exponent include an evaluation of the effect of different solid:fluid ratios on barium dissolution. This interest was motivated by two particular factors: to ensure that dissolution was not limited by saturation of the extraction fluid, and to achieve an *in vitro* environment that was more similar to the solid:fluid ratio that could be achieved under physiologic conditions in a toddler (based on an assumption of soil consumption rates of ~80 mg/day and gastric fluid production of 1 L/day, yielding a solid:fluid ratio of 1:12,500; additional discussion is provided in Axiom 2005 and Richardson 2006). Therefore, the effects of solid:fluid ratio on the dissolution of barium was investigated over a range from 1:100 to 1:10,000. Initial efforts using purified barite were conducted at five different ratios (Exponent 2005, Appendix B). Based on this information from the preliminary phases using purified barite, extraction of soils was conducted at three different soil:fluid ratios spanning the same range.

Effect of pH on Extraction of Barium

A final modification of the RBALP method that was investigated as part of this research was an evaluation of the effect of pH on the dissolution of barium from barite. It has been well established that the dissolution of other metals from soil or other solid matrices is influenced by the pH of the receptor fluid (Ruby et al. 1996). The RBALP method was optimized to evaluate dissolution under the physiologic conditions of a human child. However, for barium and other soils, potential exposures by ecological receptors can be a significant consideration. The gastrointestinal milieu of common sentinel species of ecological receptors such as the shrew includes a higher pH than found in humans. Recent research indicates that, for lead, extractions conducted at pH 4.5 are representative of ecological receptors and correlate best with data from *in vivo* studies of the relative oral bioavailability of metals from soil (SERDP 2005). In order to address this issue, one set of extractions was conducted at pH 4.5, to allow comparison against data collected at a lower pH.

Phased Approach

At the request of CAPP, a phased approach was implemented in this research. Prior to testing of site soils, preliminary studies were performed using purified barium sulfate ($BaSO_4$) as the extracted substrate. Soils were not investigated as a research substrate until final phases of the research program. Phases I and II focused on using purified barium sulfate to assess the influence of the solid:fluid ratio, particle size, and pH on barium dissolution.

This report provides the results of *in vitro* extraction testing. The Phase I and Phase II work is described below, followed by a full description of the findings of the soil extraction efforts.

Phase I

As specified by the proposed work plan developed by CAPP (Axiom 2005), extraction testing with purified barium sulfate was conducted at five discrete solid:fluid ratios:

- 1:100
- 1:300
- 1:1,000
- 1:3,000
- 1:10,000

This phase of work was undertaken to assess whether the bioaccessibility extractions were accurately assessing time-dependent dissolution, or the results of the extraction tests were controlled by the saturation limit of the extraction fluid. Because the volume of the containers used for extraction is constrained to a size that will fit in the extraction apparatus, the different solid:fluid ratios were achieved by holding the fluid volume constant and varying the mass of the solid extracted.

Phase II

As specified in the work plan (Axiom 2005), Phase II efforts focused on using purified barium sulfate to assess the influence of particle size on the dissolution of barium in the extraction fluid. The purified barium sulfate used in the extractions is specified as having a distribution of grain sizes from 45 to 325 μm (AlfaAesar 2005). In the Phase I extractions, this bulk material was used for extractions at all solid:fluid ratios evaluated. Under Phase II, two discrete particle size fractions were conducted at a solid:fluid ratio of 1:10,000 (the highest ratio evaluated). The particle size ranges evaluated were particles from 180 to 250 μm, and particles <38 μm after grinding and sieving of the bulk material.

Phase III: Extraction Testing of Soils

The results of the Phase I and II testing, using purified barite, were used to inform the design of the soil extraction test. Specifically, extraction testing of soils included evaluation of three different soil particle sizes (<2 mm, or "bulk" fraction, the particle size fraction from <250 μm to 38 μm, and the <38-μm fraction), and were extracted at three different solid:fluid ratios (1:100, 1:1,000, and 1:10,000). Additionally, the first set of soil extractions were all conducted in triplicate, to assess variability in the extraction results. Details of the extraction process and the results are discussed below.

Materials

The soils evaluated in this study were collected from four discrete site locations in Canada. All soil samples were supplied to Exponent by Axiom Environmental, and arrived under chain of custody. Each soil was designated with a "site number" but with no additional identifiers with regard to the specific source of the soil. Following receipt, all soils were air dried and screened to target particle sizes.

Results from Phase I and II Investigations

Results from the first two phases of the investigation were compiled and evaluated prior to advancing to evaluation of barium dissolution from barite-containing soils. Data from each extraction, including information regarding the solid:fluid ratio, the grain size evaluated, mass of barium sulfate extracted and corresponding mass of barium extracted, pH, concentration and mass of barium in the extraction fluid, and calculated barium bioaccessibility, are provided in Table 1. Findings indicate that the solid:fluid ratio has a strong influence on the dissolution of barium from the barium sulfate evaluated in these extraction studies. Calculated bioaccessibility of barium from barium sulfate (i.e., the mass of barium in the extraction fluid divided by the mass of barium in the solid matrix extracted) was consistent across the replicate extractions conducted, but varied by almost two orders of magnitude across the solid:fluid ratios evaluated. Figure 1 provides a graphic representation of the data, and suggests that the dissolution of the barium from barium sulfate demonstrates a consistent relation with solid:fluid ratio, and that the amount of barium dissolved at the lowest solid:fluid ratio of 1:100 is close to the solubility limit (approximated as the mass of barium dissolved when the extraction process was allowed to continue over 43.5 hours).

In developing the *in vitro* method used in these extractions, researchers found that the dissolution of lead from soils was dependent on the solid:fluid ratio, with higher dissolution per unit mass associated with the lower ratios (Medlin 1997). Specifically, over a 4-fold range in the soil:fluid ratio (i.e., from 1:125 to 1:500), Medlin reports an 11% change in the calculated lead bioaccessibility (changing from about 69% to 80% bioaccessibility across the ratios tested). Therefore, our results are consistent with what is understood about dissolution kinetics and with experience in testing of lead during development of the RBALP *in vitro* extraction method.

Also compiled in Table 1 are data regarding the influence of particle size on the calculated bioaccessibility of barium from the barium sulfate. This analysis suggests good reproducibility across replicate extractions, but only a very small difference (probably within the range of analytical error) between the solubility of barium sulfate from the two different discrete particle size ranges evaluated. Interestingly, the calculated bioaccessibility for these two, smaller, particle size fractions was lower than the calculated bioaccessibility achieved from extraction of the bulk barium sulfate material. The source of this difference is unknown, but could be analytical variability, or it may indicate that the bulk material was more soluble.

Finally, Table 1 shows the compiled data from extractions conducted at the higher pH than the standard method. Specifically, an extraction was conducted at pH 4.5, and these data can be compared to the other extractions conducted at the solid:fluid ratio of 1:1000 to assess the influence of pH on the dissolution of barium. As expected, barium was less soluble at the higher pH, indicating that barium in barium sulfate may be less bioavailable to ecological receptors, such as small mammals, that have less aggressive conditions in their gastrointestinal tract.

Results from Phase III Investigations

Soil extractions were conducted in three stages. The staged process was required due to spurious results and the identification of background contamination of the extraction fluid due to the initial use of barium-containing filters. This source of background was confirmed, barium-free filters were obtained, and subsequent extractions were performed with barium-free filters. Data and correspondence regarding the early stages of the soil extraction process are presented in Appendix B. The final results, having resolved the contamination issues, are discussed below.

Four site soils with high concentrations of barium from barite contamination, and one "control" soil from an uncontaminated area were evaluated for barium bioaccessibility. For three of the soils evaluated (i.e., Site #1, Site #2, and the control soil), triplicate bioaccessibility extractions were conducted for the three particle size ranges and at the three soil:fluid ratios described above. For soils from Site #3 and Site #4, only the smallest particle size fraction (i.e., <38 μm) was evaluated, and not all extractions were conducted in replicates. However, for all soils, the full suite of soil:fluid ratios was evaluated.

All data derived from the extractions of the site soils are presented in Tables 2a through 2c. Figures 2a through 5b provide corresponding graphical representations of the data. Table 3 provides quality assurance data collected during the extraction process. Figure 6 presents a synthesis of the full data set, across all soils.

The reported concentrations of barium in these soils varied depending on whether the total barium concentrations were measured using a standard soil digestion (i.e., USEPA Method 3050B) or XRF. For soils in the low range of total barium concentrations (i.e., 1000 mg/kg to 5000 mg/kg), the USEPA Method 3050B digestion method resulted in reported total barium concentrations in the soil that were slightly greater than or equal to the results achieved with XRF. At higher total barium concentrations, the USEPA Method 3050B soil digestion resulted in significantly lower total barium values, relative to the results achieved with XRF analysis. Although the Method 3050B soil digestion is specified as an appropriate method for "total metals in soils," there are known exceptions documented within the method, including barium, for which this method can yield low results (ESG 2003; Medved et al. 1998). Because the extraction fluid used in bioaccessibility testing extracted more barium than the soil digestion in USEPA Method 3050B, bioaccessibility values calculated based on the 3050B results for barium concentration in the soil resulted in the spurious reporting of bioaccessibility values greater than 100%. Obviously, the bioaccessible fraction of barium from barite-contaminated soils cannot exceed the total amount of barium in the soils. Bioaccessibility calculated based on the XRF results, which better represent total barium for the high-barium soils, produced values that are all below 100%.

Regardless of analytical method, barium concentrations in the contaminated site soils ranged considerably higher than barium concentrations in the one "control" soil tested. Results of the barite bioaccessibility testing of Canada soils are presented in Tables 2a–2c. Data incorporated into these tables include the specific soil evaluated, the particle size, soil:fluid ratio, barium concentrations in each particle size (by EPA Method 3050B and XRF), concentration of barium in the extraction fluid, and the calculated bioaccessibility (based on both methods for assessing total barium concentrations in the soil). For each of the Figures 2 through Figure 5, figure "a" presents the data corresponding to the results from total barium concentrations based on EPA Method 3050B, and figure "b" depicts results corresponding to total barium concentrations derived from XRF.

Effect of Particle Size

Results indicate that, for all soils tested at different particle sizes, the soil particle size demonstrated a limited effect on the calculated bioaccessibility. Generally, the particle size influenced the calculated bioaccessibility by less than 25%, but variation in bioaccessibility with particle size varied by about 2-fold in some instances. Interestingly, the calculated bioaccessibility is directly related to particle size for some soils, and inversely related particle size for others. Also interesting is the observation that, for soils where particle size had a larger influence on calculated bioaccessibility, there is no clear trend across the different soil:fluid ratios, and the influence of particle size may be inversely related to bioaccessibility if based on USEPA Method 3050B soil digestion, but unrelated to bioaccessibility if based on XRF concentrations of barium in the soil (for example see data for Site #2 Test Soil in figures 3a and 3b). The data do not support a definitive determination of these relations.

Effect of Soil:Fluid Ratio

With each of the soils tested, extractions were conducted at the three target soil:fluid ratios of 1:100, 1:1,000, and 1:10,000. Data for each triplicate extraction at each soil:fluid ratio are presented in Tables 2a–c and graphically in Figures 2a–5b. These results indicate that the calculated bioaccessibility varies considerably, depending on the soil:fluid ratio used in the extraction. The incremental increase in the relative dissolution of barium per unit mass is greater with the change in soil:fluid ratio from 1:100 to 1:1000. However, there is also a consistent increase in dissolution at the highest soil:fluid ratio of 1:10,000. Specifically, there is a 5- to 10-fold increase in the calculated bioaccessibility for site soils with the change from 1:100 to 1:1,000, whereas the change in calculated bioaccessibility remains constant or increases by up to 3-fold with the change in soil:fluid ratio of 1:1,000 to 1:10,000.

Discussion

For lead, we know that the *in vitro* bioaccessibility results correlate well with the relative oral bioavailability of lead as measured in animals, and that the correlation of the *in vitro* bioaccessibility to *in vivo* RBA is near unity for extractions conducted at a soil:fluid ratio of 1:100 (Drexler and Brattin 2007).

The results from testing of barite-contaminated soils demonstrate that site-specific factors appear to affect the bioaccessibility of barium from soils. This is demonstrated by differences in bioaccessibility among the soils tested, varying by as much as 10-fold (based on a comparison of data from the different soils), with soil particle size and soil:fluid ratio held constant.

Soil particle size and soil:fluid ratio both can affect the bioaccessibility of barium from soils, but the influence of particle size is smaller and inconsistent with regard to its relation to bioaccessibility (i.e., for some soils, it demonstrated a direct relation with bioaccessibility, whereas an inverse relation is demonstrated for other soils). Soil:fluid ratio has a more consistent effect on calculated bioaccessibility, with higher bioaccessibility indicated at the higher soil:fluid ratios. In the absence of data from *in vivo* research, against which to calibrate the extraction method, it is unknown which soil:fluid ratio is more representative of the relative oral bioavailability of barium from barite-contaminated soils.

These factors of soil particle size and soil:fluid ratio have long been known to influence the dissolution of metals from soils. In addition to these expected factors, the results of this extraction testing on four site soils contaminated with barite points out the unique importance of selecting appropriate analytical methods for characterizing concentrations of barium in soil. In order to be relevant for making site-specific decisions regarding risks or remediation, measurements of bioaccessibility (or bioavailability, if conducted in an animal model) of site soils should be carried out with reference to the method that has been used to characterize barium concentrations at the site of concern: If the site has been characterized using the XRF method to assess the concentration of barium in soils, then the bioaccessibility estimates should also be conducted based on the XRF methods in soils. If barium concentrations in the soils have been characterized in tandem with other metals, using standard "wet lab" analytical methods such as USEPA Method 3050B, then bioaccessibility values should be calculated based on similar data, with the understanding that the soluble fraction of soil barium in the simulated gastric fluid may exceed solubility in the soil digestion method, resulting in the apparently spurious findings of bioaccessibility values exceeding 100%.

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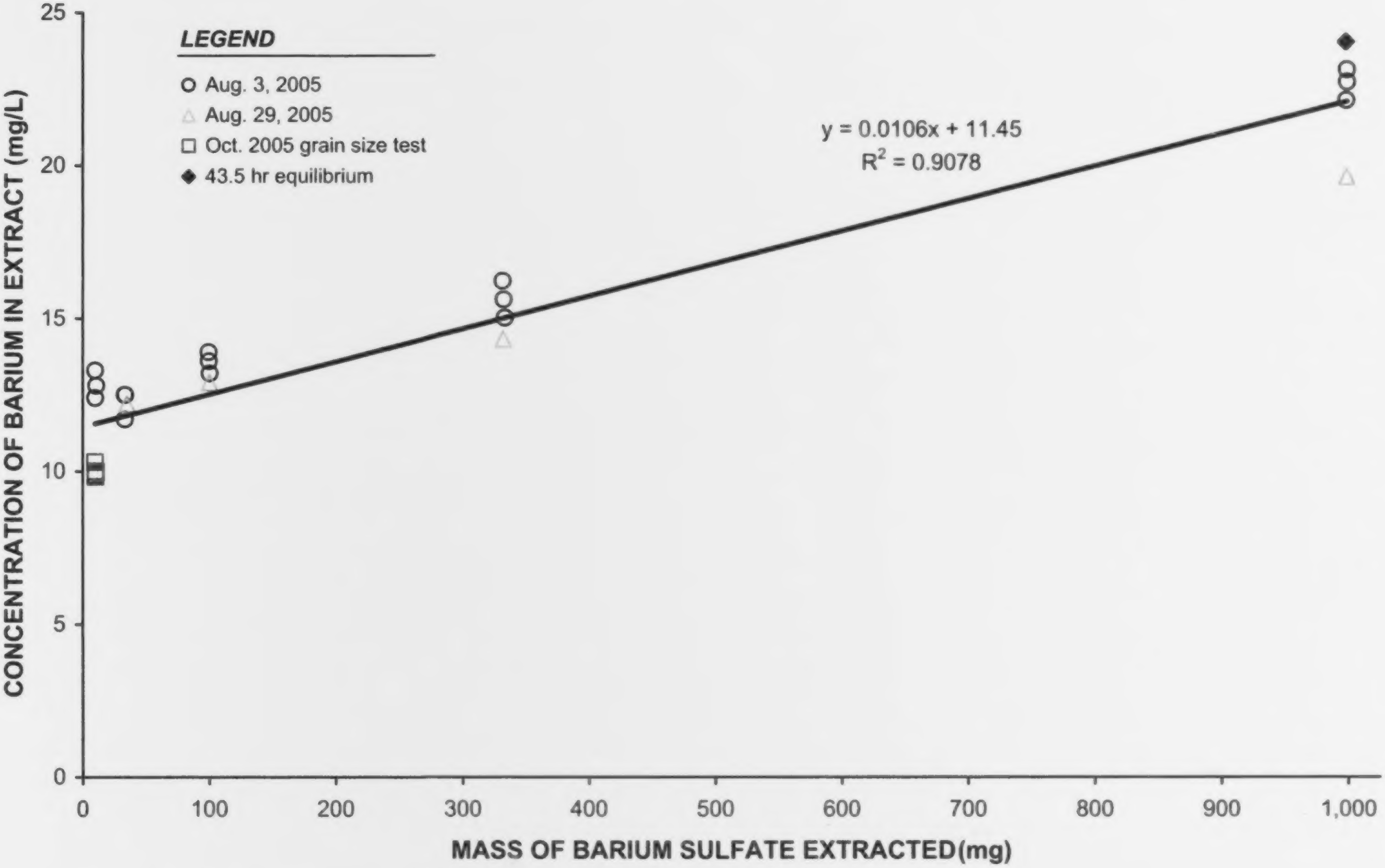
Figures



Note: Trendline incorporates all data except the 43.5-hr equilibrium sample.

Figure 1. Mass of barium sulfate extracted vs. barium concentration in extract

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Barite_InVitro.xls Chart_OneTrendline 11/16/2005 (9:57 AM)

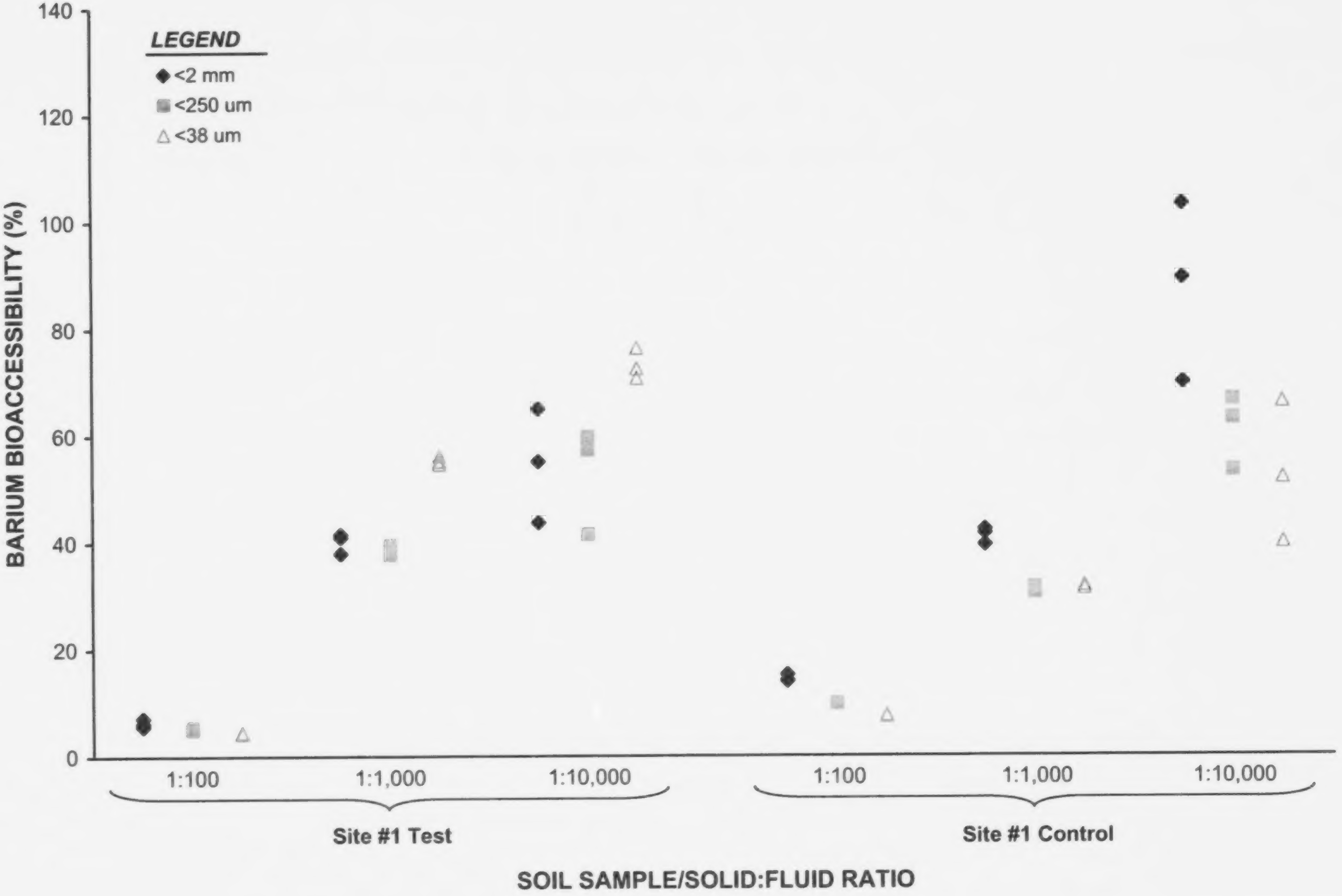

Figure 2a. *In vitro* barium bioaccessibility of Canada soils for Site #1 calculated using soil concentrations from Method 3050B

Barite_Soils_InVitro04.xls Figure 2a 3/1/2007 (4:26 PM)

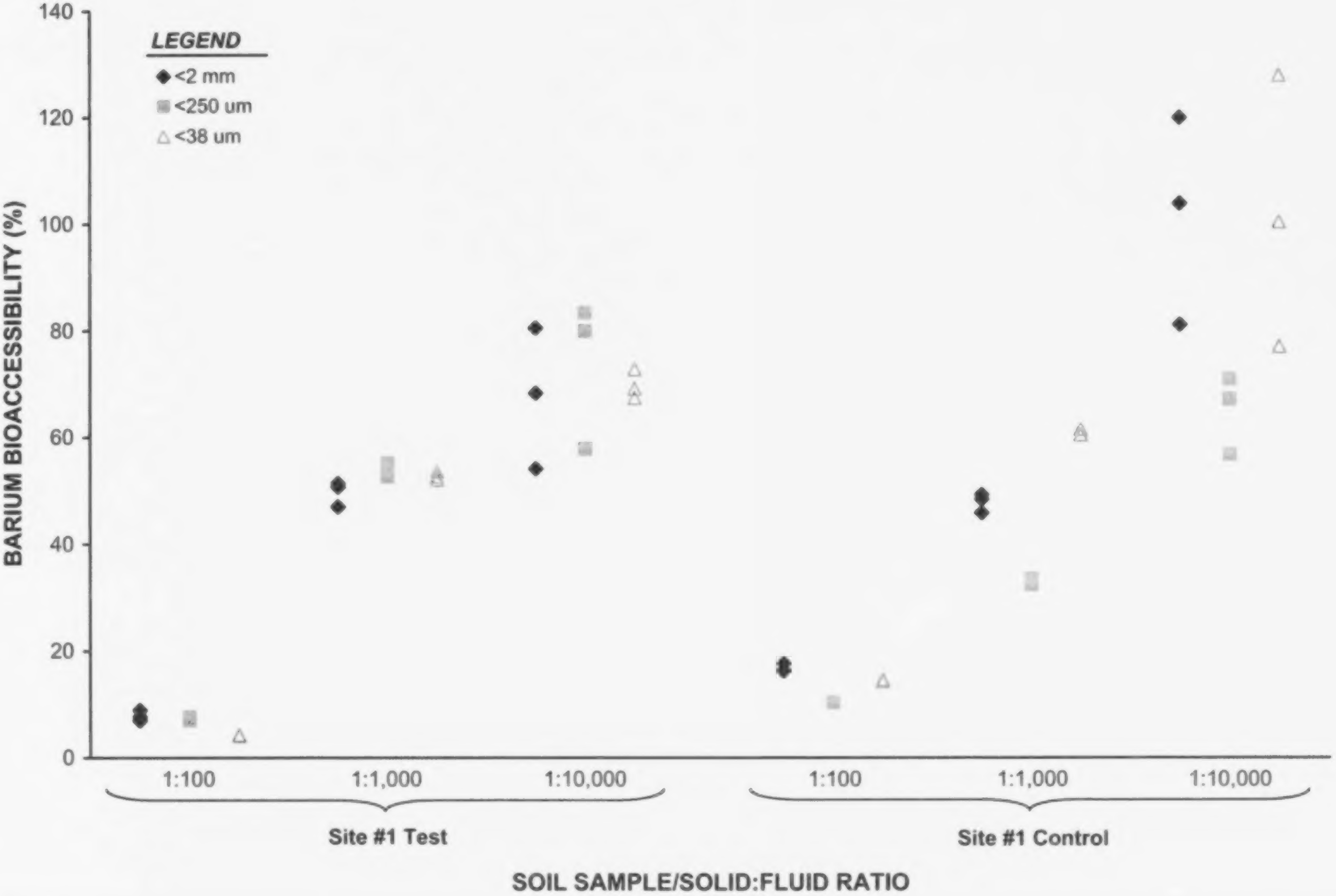

Figure 2b. *In vitro* barium bioaccessibility of Canada soils for Site #1 calculated using soil concentrations from XRF

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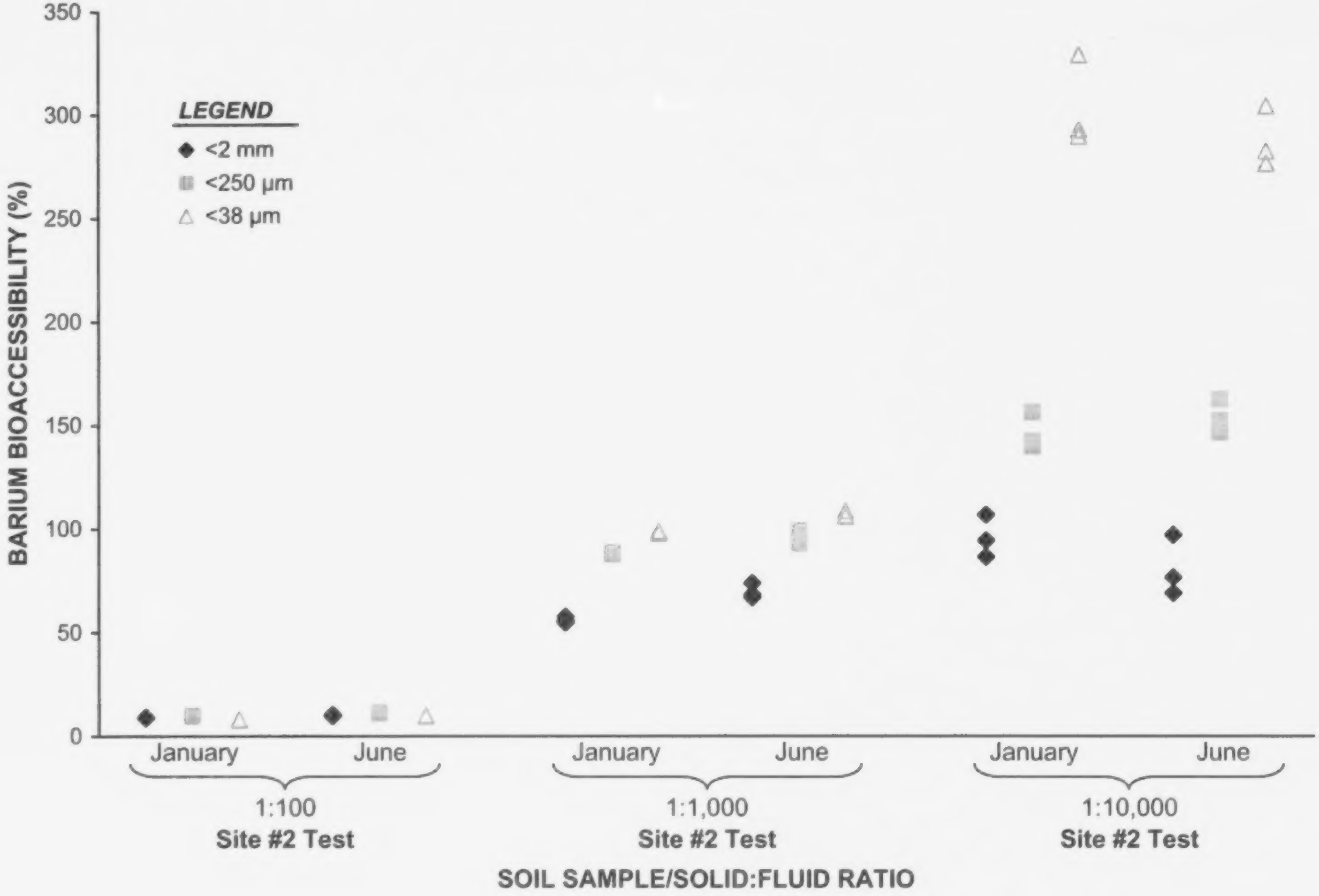

Figure 3a. *In vitro* barium bioaccessibility of Canada soils for Site #2 Test — January and June 2006 data calculated using soil concentrations from Method 3050B

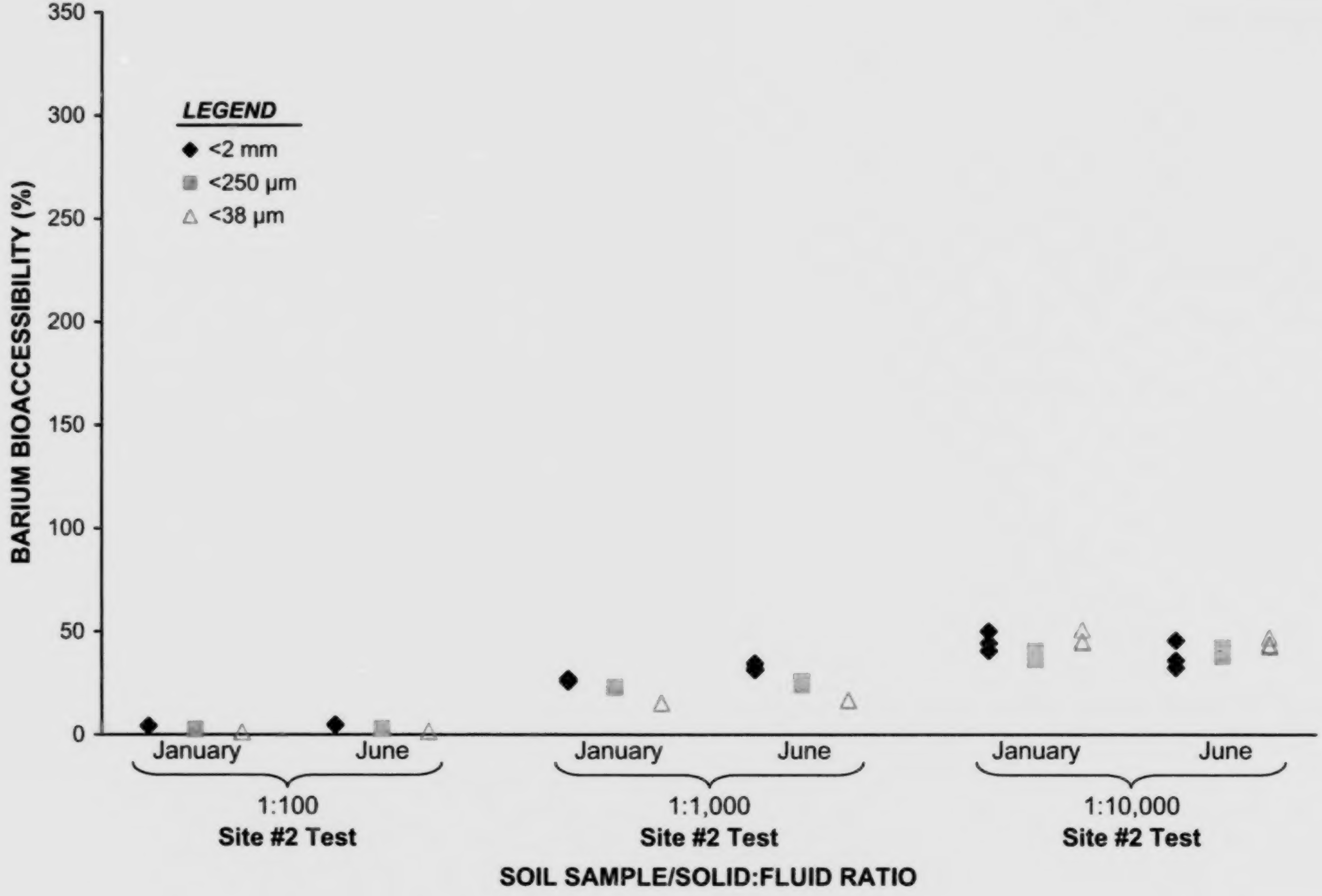

Figure 3b. *In vitro* barium bioaccessibility of Canada soils for Site #2 Test — January and June 2006 data calculated using soil concentrations from XRF

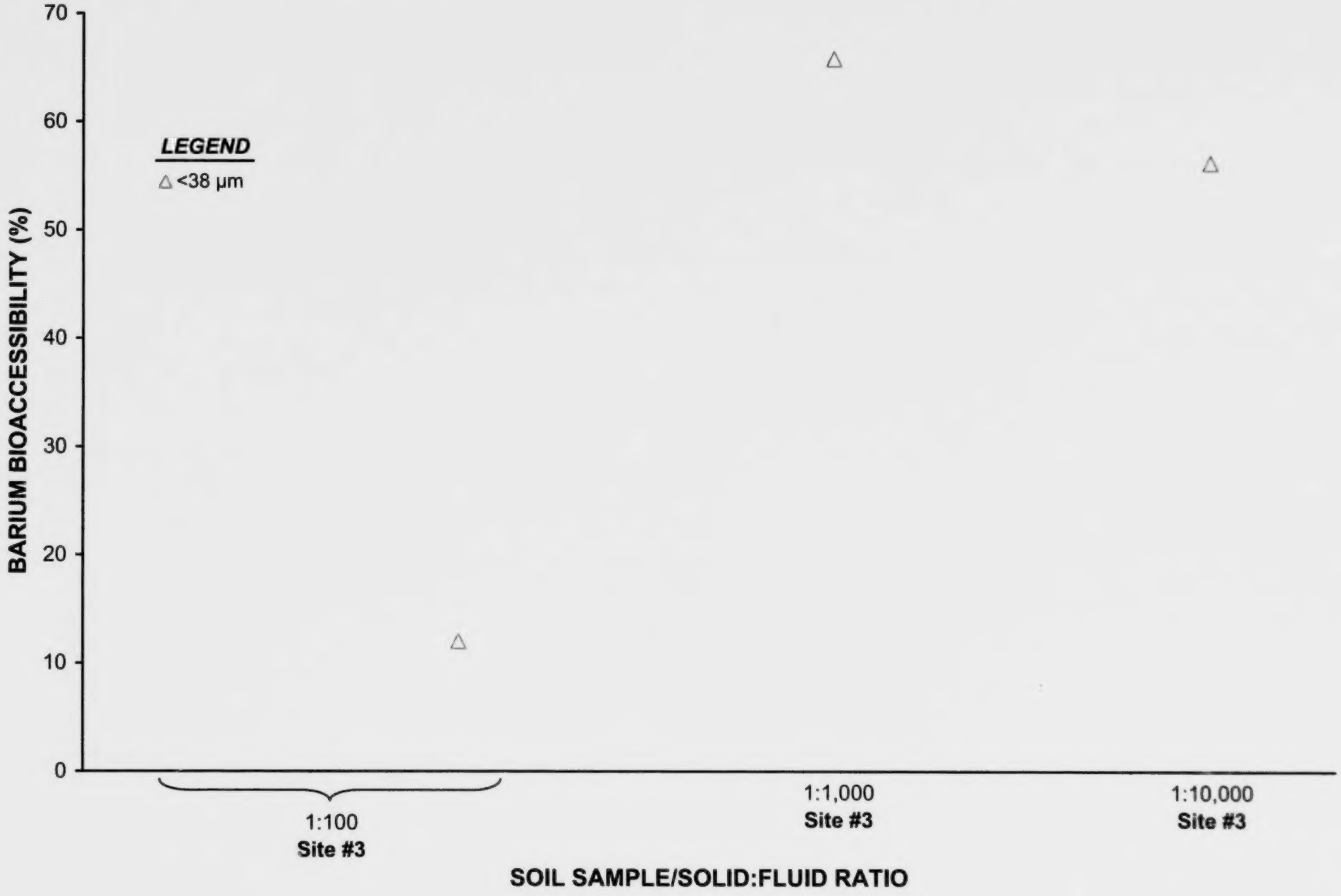

Figure 4a. *In vitro* barium bioaccessibility of Canada soils for Site #3 calculated using soil concentrations from Method 3050B

Barite_Soils_InVitro04.xls Figure 4a 3/1/2007 (4:26 PM)

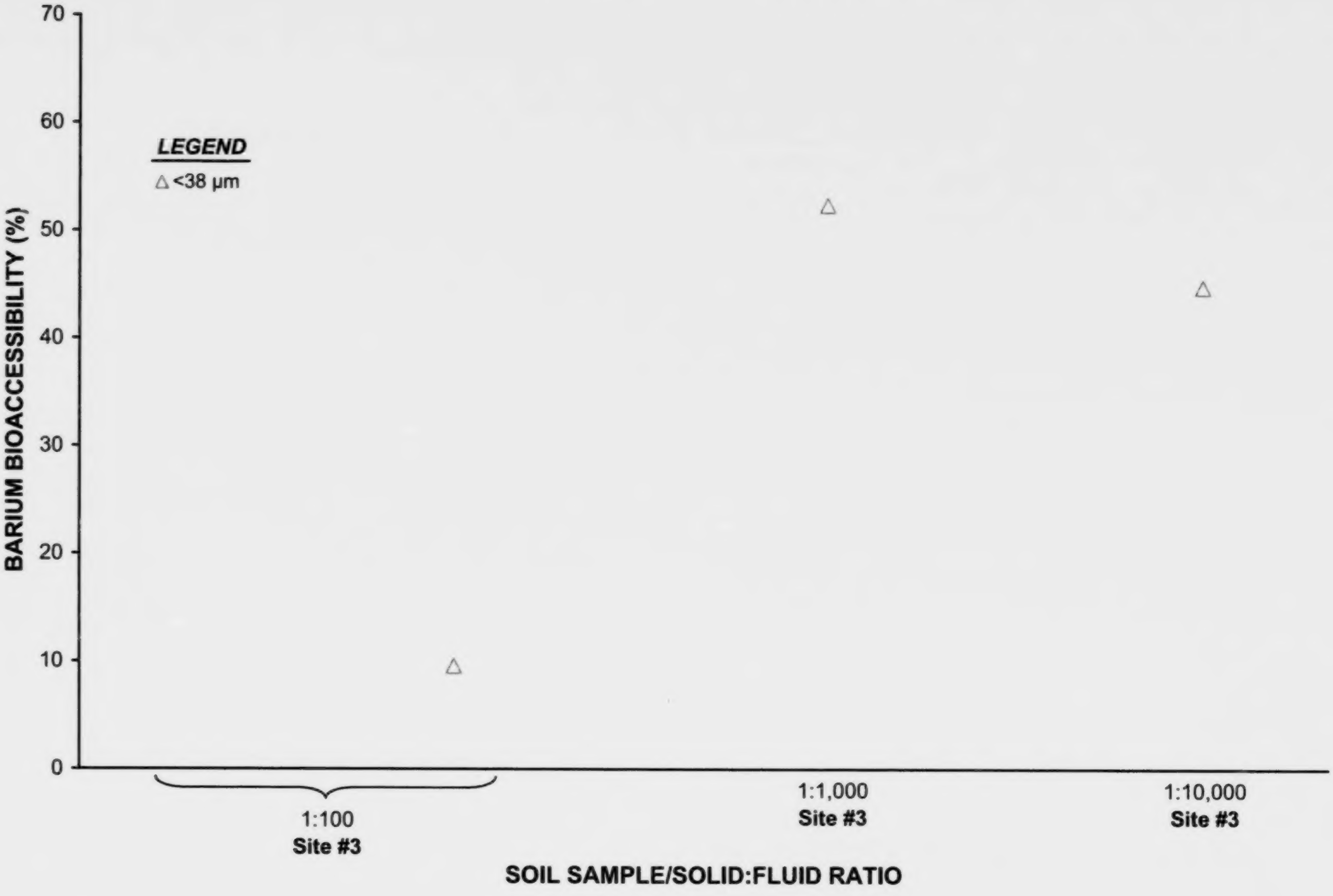

Figure 4b. *In vitro* barium bioaccessibility of Canada soils for Site #3 calculated using soil concentrations from XRF

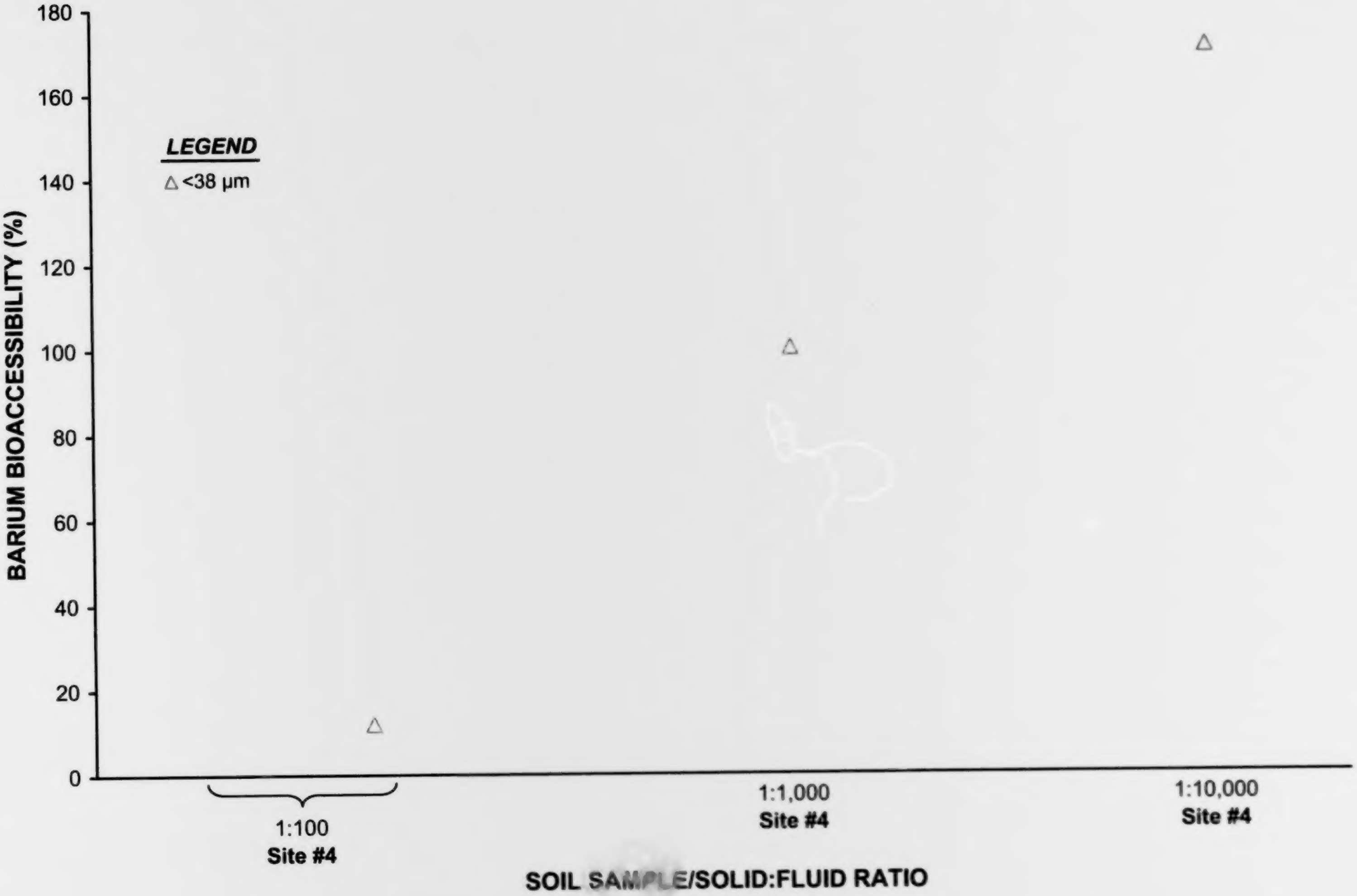

Figure 5a. *In vitro* barium bioaccessibility of Canada soils for Site #4 calculated using soil concentrations from Method 3050B

Barite_Soils_InVitro04.xls Figure 5a 3/1/2007 (4:26 PM)

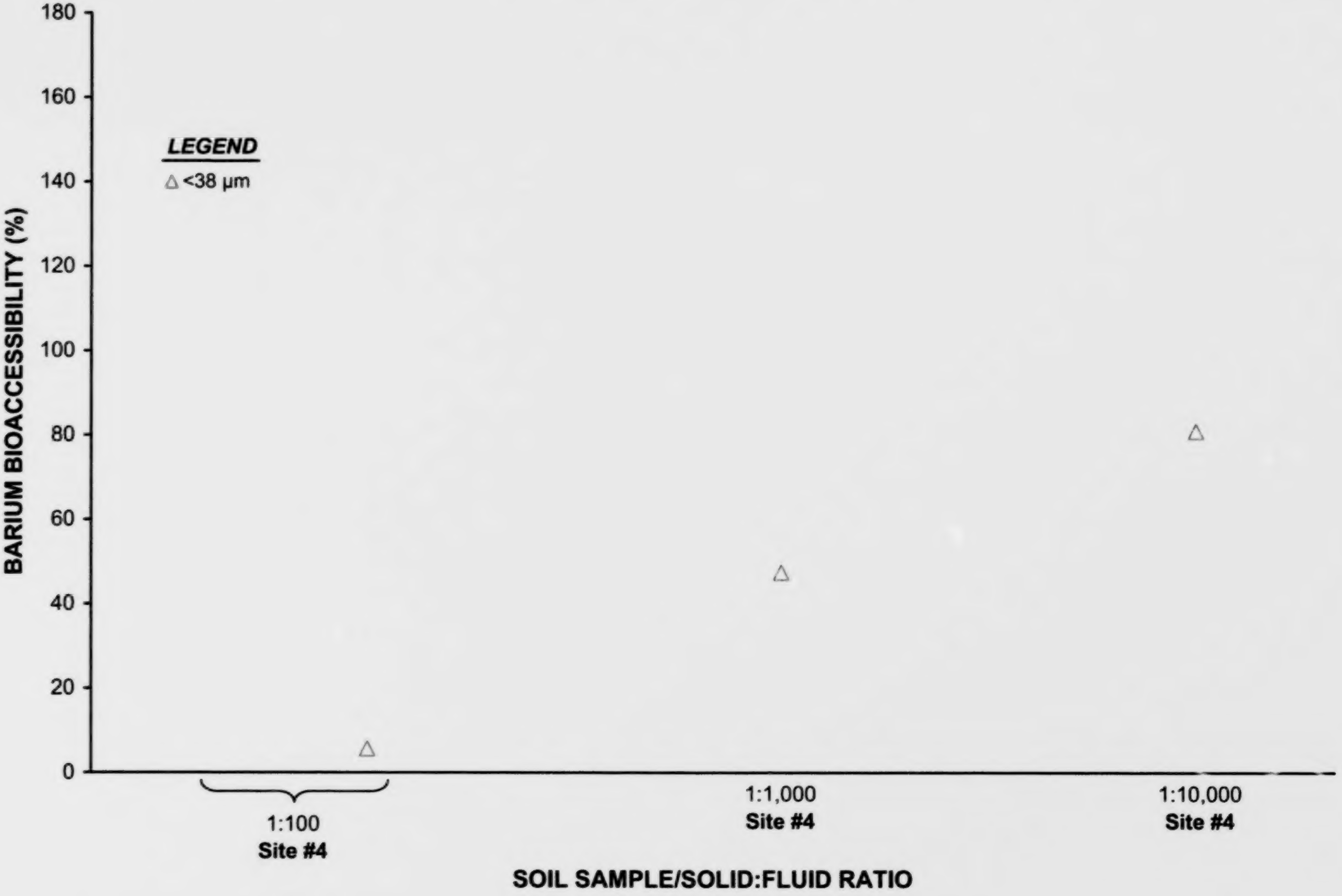

Figure 5b. *In vitro* barium bioaccessibility of Canada soils for Site #4 calculated using soil concentrations from XRF

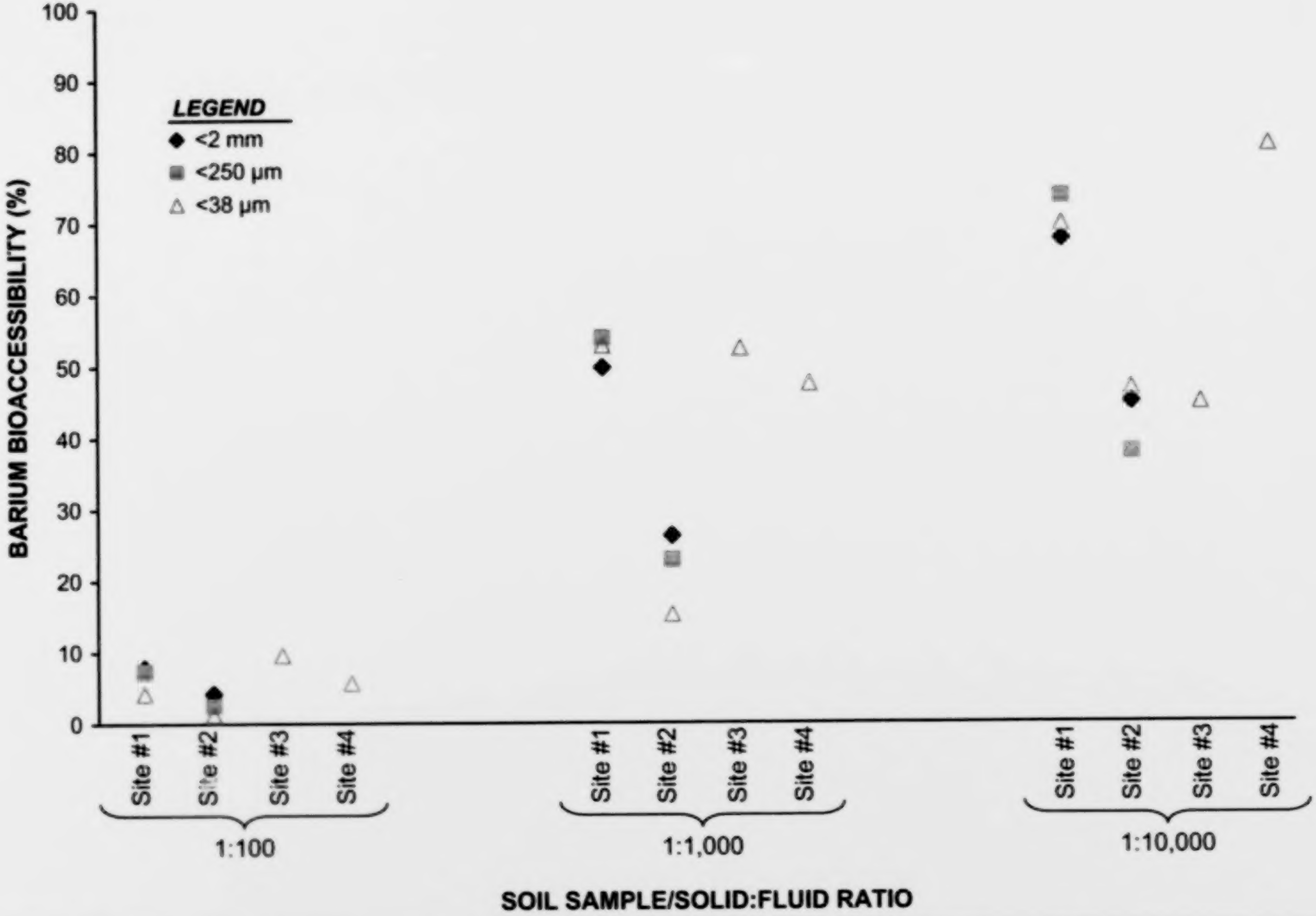

Figure 6. In vitro barium bioaccessibility of Canada soils for all sites calculated using soil concentrations from XRF

Barite_Soils_InVitro04 (2).xls Figure 5 3/15/2007 (11:28 AM)

Tables

Table 1. Results from *in vitro* bioaccessibility testing of barite mixtures

Barite Ratio Fluid : Solid	Sample Number	Grain Size (µm)	Date	Mass of $BaSO_4$ Extracted (mg)	Mass of Barium Extracted[a] (mg)	Initial pH (s.u.)	Barium Conc. in Extract (mg/L)	Volume of Extract (L)	Mass of Barium in Extract (mg)	Barite (as barium) Bioaccess-ibility (%)
100:1	AEB001	45–325[b]	8/3/05	999.8	588.3	1.5	22.1	0.1	2.21	**0.38**
100:1	AEB002	45–325[b]	8/3/05	1,000.4	588.6	1.5	22.7	0.1	2.27	**0.39**
100:1	AEB003	45–325[b]	8/3/05	1,000.4	588.6	1.5	23.1	0.1	2.31	**0.39**
100:1	AEB022	45–325[b]	8/29/05	999.9	588.3	1.5	19.6	0.1	1.96	**0.33**
300:1	AEB004	45–325[b]	8/3/05	332.5	195.6	1.5	16.2	0.1	1.62	**0.83**
300:1	AEB005	45–325[b]	8/3/05	334.2	196.6	1.5	15.0	0.1	1.50	**0.76**
300:1	AEB006	45–325[b]	8/3/05	333.4	196.2	1.5	15.6	0.1	1.56	**0.80**
300:1	AEB023	45–325[b]	8/29/05	332.7	195.8	1.5	14.3	0.1	1.43	**0.73**
1,000:1	AEB007	45–325[b]	8/3/05	99.5	58.5	1.5	13.9	0.1	1.39	**2.4**
1,000:1	AEB008	45–325[b]	8/3/05	100.5	59.1	1.5	13.2	0.1	1.32	**2.2**
1,000:1	AEB009	45–325[b]	8/3/05	99.8	58.7	1.5	13.6	0.1	1.36	**2.3**
1,000:1	AEB024	45–325[b]	8/29/05	100	58.8	1.5	12.9	0.1	1.29	**2.2**
3,000:1	AEB011	45–325[b]	8/3/05	33.5	19.7	1.5	11.7	0.1	1.17	**5.9**
3,000:1	AEB012	45–325[b]	8/3/05	33.7	19.8	1.5	12.5	0.1	1.25	**6.3**
3,000:1	AEB013	45–325[b]	8/3/05	33.3	19.6	1.5	12.5	0.1	1.25	**6.4**
3,000:1	AEB025	45–325[b]	8/29/05	34.8	20.5	1.5	12.2	0.1	1.22	**6.0**
10,000:1	AEB014	45–325[b]	8/3/05	10.8	6.35	1.5	12.8	0.1	1.28	**20**
10,000:1	AEB015	45–325[b]	8/3/05	10.0	5.88	1.5	12.4	0.1	1.24	**21**
10,000:1	AEB016	45–325[b]	8/3/05	10.0	5.88	1.5	13.3	0.1	1.33	**23**
10,000:1	AEB031	180–250	10/4/05	9.8	5.77	1.5	10.3	0.1	1.03	**18**
10,000:1	AEB032	180–250	10/4/05	10.2	6.00	1.5	9.90	0.1	0.99	**16**
10,000:1	AEB033	180–250	10/4/05	10.3	6.06	1.5	9.99	0.1	1.00	**16**
10,000:1	AEB034	<38	10/4/05	10.1	5.94	1.5	9.82	0.1	0.98	**17**
10,000:1	AEB035	<38	10/4/05	10.1	5.94	1.5	10.0	0.1	1.00	**17**
10,000:1	AEB036	<38	10/4/05	10.0	5.88	1.5	9.86	0.1	0.99	**17**
100:1[c]	AEB037	45–325[b]	10/6/05	999.5	588.1	1.5	24.0	0.1	2.40	**0.41**
1,000:1	AEB018	45–325[b]	8/3/05	99.9	58.8	4.5	9.29	0.1	0.93	**1.6**
1,000:1	AEB019	45–325[b]	8/3/05	100.1	58.9	4.5	10.2	0.1	1.02	**1.7**
1,000:1	AEB020	45–325[b]	8/3/05	100.1	58.9	4.5	10.1	0.1	1.01	**1.7**

[a] Calculated as (Mass of $BaSO_4$) × (M.W. Ba / M.W. $BaSO_4$) where;

M.W. Ba = 137.33

M.W. $BaSO_4$ = 233.4

[b] Grain size as specified by AlfaAesar for Puratronic 99.998% (metals basis) barium sulfate

[c] This extraction procedure run for 43.5 hours.

Table 2a. Barium bioaccessibility results from Canada soils

		Barium	Barium Concentration In Soil		Mass of Soil	Mass of Barium in Soil Extracted			Post-Test	Barium Conc. in	Volume of	Mass of Barium	Barium Bioaccessibility		Mean Barium Bioaccessibility	
Soil ID	Grain Size	Ratio Solid : Fluid	3050B[a] (mg/kg)	XRF[b] (mg/kg)	Tested (g)	3050B (mg)	XRF (mg)	Extraction Date	pH (s.u.)	Extract[c] (mg/L)	Extract (L)	in Extract (mg)	3050B (%)	XRF (%)	3050B (%)	XRF (%)
Site #1 Test	<2 mm	1:100	3,849	3,104	1.0078	3.879	3.128	1/17/06	1.66	2.178	0.100	0.218	5.6	7.0		
Site #1 Test	<2 mm	1:100	3,849	3,104	1.0040	3.864	3.116	1/17/06	1.62	2.770	0.100	0.277	7.2	8.9		
Site #1 Test	<2 mm	1:100	3,849	3,104	1.0046	3.867	3.118	1/17/06	1.61	2.376	0.100	0.238	6.1	7.6	6.3	7.8
Site #1 Test	<250 µm	1:100	4,776	3,418	1.0075	4.812	3.444	1/17/06	1.58	2.393	0.100	0.239	5.0	6.9		
Site #1 Test	<250 µm	1:100	4,776	3,418	1.0069	4.809	3.442	1/17/06	1.59	2.605	0.100	0.261	5.4	7.6		
Site #1 Test	<250 µm	1:100	4,776	3,418	1.0046	4.798	3.434	1/17/06	1.59	2.555	0.100	0.256	5.3	7.4	5.2	7.3
Site #1 Test	<38 µm	1:100	5,084	5,328	1.0011	5.090	5.334	1/17/06	1.61	2.271	0.100	0.227	4.5	4.3		
Site #1 Test	<38 µm	1:100	5,084	5,328	1.0076	5.123	5.368	1/17/06	1.65	2.207	0.100	0.221	4.3	4.1		
Site #1 Test	<38 µm	1:100	5,084	5,328	1.0058	5.113	5.359	1/17/06	1.63	2.236	0.100	0.224	4.4	4.2	4.4	4.2
Site #1 Test	<2 mm	1:1,000	3,849	3,104	0.1057	0.407	0.328	1/17/06	1.45	1.664	0.100	0.166	41	51		
Site #1 Test	<2 mm	1:1,000	3,849	3,104	0.1027	0.395	0.319	1/17/06	1.46	1.638	0.100	0.164	41	51		
Site #1 Test	<2 mm	1:1,000	3,849	3,104	0.1028	0.396	0.319	1/17/06	1.45	1.499	0.100	0.150	38	47	40	50
Site #1 Test	<250 µm	1:1,000	4,776	3,418	0.1077	0.514	0.368	1/17/06	1.45	1.935	0.100	0.194	38	53		
Site #1 Test	<250 µm	1:1,000	4,776	3,418	0.1060	0.506	0.362	1/17/06	1.47	1.998	0.100	0.200	39	55		
Site #1 Test	<250 µm	1:1,000	4,776	3,418	0.1015	0.485	0.347	1/17/06	1.45	1.864	0.100	0.186	38	54	39	54
Site #1 Test	<38 µm	1:1,000	5,084	5,328	0.1050	0.534	0.559	1/17/06	1.46	2.912	0.100	0.291	55	52		
Site #1 Test	<38 µm	1:1,000	5,084	5,328	0.1053	0.535	0.561	1/17/06	1.45	3.003	0.100	0.300	56	54		
Site #1 Test	<38 µm	1:1,000	5,084	5,328	0.1062	0.540	0.566	1/17/06	1.45	2.977	0.100	0.298	55	53	55	53
Site #1 Test	<2 mm	1:10,000	3,849	3,104	0.0123	0.047	0.038	1/17/06	1.60	0.307	0.100	0.031	65	80		
Site #1 Test	<2 mm	1:10,000	3,849	3,104	0.0143	0.055	0.044	1/17/06	1.59	0.303	0.100	0.030	55	68		
Site #1 Test	<2 mm	1:10,000	3,849	3,104	0.0134	0.052	0.042	1/17/06	1.58	0.225	0.100	0.023	44	54	55	68
Site #1 Test	<250 µm	1:10,000	4,776	3,418	0.0115	0.055	0.039	1/17/06	1.57	0.227	0.100	0.023	41	58		
Site #1 Test	<250 µm	1:10,000	4,776	3,418	0.0140	0.067	0.048	1/17/06	1.57	0.382	0.100	0.038	57	80		
Site #1 Test	<250 µm	1:10,000	4,776	3,418	0.0136	0.065	0.046	1/17/06	1.57	0.387	0.100	0.039	60	83	53	74
Site #1 Test	<38 µm	1:10,000	5,084	5,328	0.0121	0.062	0.064	1/17/06	1.59	0.445	0.100	0.045	72	69		
Site #1 Test	<38 µm	1:10,000	5,084	5,328	0.0110	0.056	0.059	1/17/06	1.57	0.426	0.100	0.043	76	73		
Site #1 Test	<38 µm	1:10,000	5,084	5,328	0.0131	0.067	0.070	1/17/06	1.57	0.470	0.100	0.047	71	67	73	70

[a] Soil values are an average of triplicate split samples using Method 3050B.

[b] Soil XRF values.

[c] Concentrations have been corrected for filter contamination. See Table 2 and text for details.

Table 2b. Barium bioaccessibility results from Canada soils, June 2006 data

			Barium Concentration In Soil		Mass of Soil	Mass of Barium in Soil Extracted			Post-Test	Barium Conc. in	Volume of	Mass of Barium	Barium Bioaccessibility	
Soil ID	Grain Size	Barium Ratio Solid : Fluid	3050B (mg/kg)	XRF (mg/kg)	Tested (g)	3050B (mg)	XRF (mg)	Extraction Date	pH (s.u.)	Extract (mg/L)	Extract (L)	in Extract (mg)	3050B (%)	XRF (%)
Site #2 Test	<2 mm	1:100	9,712	20,762	1.0015	9.727	20.794	4/26/06	1.61	9.402	0.100	0.940	9.7	4.5
Site #2 Test	<2 mm	1:100	9,712	20,762	1.0010	9.722	20.782	4/26/06	1.62	9.435	0.100	0.944	9.7	4.5
Site #2 Test	<2 mm	1:100	9,712	20,762	1.0010	9.721	20.781	4/26/06	1.62	9.965	0.100	0.997	10.3	4.8
Site #2 Test	<250 µm	1:100	8,322	32,175	1.0006	8.327	32.194	4/26/06	1.63	9.266	0.100	0.927	11.1	2.9
Site #2 Test	<250 µm	1:100	8,322	32,175	1.0007	8.327	32.196	4/26/06	1.64	9.009	0.100	0.901	11	3
Site #2 Test	<250 µm	1:100	8,322	32,175	1.0009	8.329	32.204	4/26/06	1.64	9.371	0.100	0.937	11	3
Site #2 Test	<38 µm	1:100	8,737	56,732	1.0002	8.739	56.745	4/26/06	1.64	8.583	0.100	0.858	9.8	1.5
Site #2 Test	<38 µm	1:100	8,737	56,732	1.0008	8.744	56.779	4/26/06	1.64	8.480	0.100	0.848	9.7	1.5
Site #2 Test	<38 µm	1:100	8,737	56,732	1.0000	8.737	56.732	4/26/06	1.64	8.722	0.100	0.872	10.0	1.5
Site #2 Test	<2 mm	1:1,000	9,712	20,762	0.1003	0.974	2.082	6/12/06	1.48	6.631	0.100	0.663	68	32
Site #2 Test	<2 mm	1:1,000	9,712	20,762	0.1011	0.982	2.099	6/12/06	1.48	7.231	0.100	0.723	74	34
Site #2 Test	<2 mm	1:1,000	9,712	20,762	0.1004	0.975	2.084	6/12/06	1.48	6.499	0.100	0.650	67	31
Site #2 Test	<250 µm	1:1,000	8,322	32,175	0.1007	0.838	3.241	6/12/06	1.47	7.754	0.100	0.775	93	24
Site #2 Test	<250 µm	1:1,000	8,322	32,175	0.1007	0.838	3.238	6/12/06	1.47	7.962	0.100	0.796	95	25
Site #2 Test	<250 µm	1:1,000	8,322	32,175	0.1001	0.833	3.222	6/12/06	1.47	8.231	0.100	0.823	99	26
Site #2 Test	<38 µm	1:1,000	8,737	56,732	0.1008	0.881	5.720	6/12/06	1.47	9.313	0.100	0.931	106	16
Site #2 Test	<38 µm	1:1,000	8,737	56,732	0.1009	0.882	5.725	6/12/06	1.47	9.368	0.100	0.937	106	16
Site #2 Test	<38 µm	1:1,000	8,737	56,732	0.1003	0.876	5.688	6/12/06	1.47	9.506	0.100	0.951	109	17
Site #2 Test	<2 mm	1:10,000	9,712	20,762	0.0110	0.107	0.228	6/12/06	1.49	0.734	0.100	0.073	69	32
Site #2 Test	<2 mm	1:10,000	9,712	20,762	0.0107	0.104	0.223	6/12/06	1.48	0.797	0.100	0.080	76	36
Site #2 Test	<2 mm	1:10,000	9,712	20,762	0.0103	0.100	0.214	6/12/06	1.48	0.972	0.100	0.097	97	45
Site #2 Test	<250 µm	1:10,000	8,322	32,175	0.0102	0.085	0.327	6/12/06	1.47	1.373	0.100	0.137	162	42
Site #2 Test	<250 µm	1:10,000	8,322	32,175	0.0106	0.088	0.341	6/12/06	1.47	1.290	0.100	0.129	146	38
Site #2 Test	<250 µm	1:10,000	8,322	32,175	0.0101	0.084	0.324	6/12/06	1.47	1.275	0.100	0.128	152	39
Site #2 Test	<38 µm	1:10,000	8,737	56,732	0.0102	0.089	0.577	6/12/06	1.47	2.509	0.100	0.251	282	43
Site #2 Test	<38 µm	1:10,000	8,737	56,732	0.0102	0.089	0.580	6/12/06	1.47	2.716	0.100	0.272	304	47
Site #2 Test	<38 µm	1:10,000	8,737	56,732	0.0106	0.093	0.601	6/12/06	1.49	2.557	0.100	0.256	276	43

[a] Soil values are an average of triplicate split samples using Method 3050B.

[b] Soil XRF values.

[c] Concentrations have been corrected for filter contamination. See Table 2 and text for details.

Table 2c. Barium bioaccessibility results from Canada soils

Soil ID	Grain Size	Barium Ratio Solid : Fluid	Barium Concentration In Soi 3050B[a] (mg/kg)	Barium Concentration In Soi XRF[b] (mg/kg)	Mass of Soil Tested (g)	Mass of Barium in Soil Extracted 3050B (mg)	Mass of Barium in Soil Extracted XRF (mg)	Extraction Date	Post-Test pH (s.u.)	Barium Conc. in Extract[c] (mg/L)	Volume of Extract (L)	Mass of Barium in Extract (mg)	Barium Bioaccessibility 3050B (%)	Barium Bioaccessibility XRF (%)	Mean Barium Bioaccessibility 3050B (%)	Mean Barium Bioaccessibility XRF (%)
Site #3	<38 µm	1:100	9,185	11,537	1.0135	9.309	11.693	10/13/06	1.57	11.120	0.100	1.112	12	10		
Site #3	<38 µm	1:100	9,185	11,537	1.0124	9.299	11.680	10/13/06	1.58	11.050	0.100	1.105	12	9		
Site #3	<38 µm	1:100	9,185	11,537	1.0036	9.218	11.578	10/13/06	1.58	11.200	0.100	1.120	12	10	12	10
Site #3	<38 µm	1:1,000	9,185	11,537	0.1100	1.011	1.270	10/13/06	1.52	6.644	0.100	0.664	66	52	66	52
Site #3	<38 µm	1:10,000	9,185	11,537	0.0162	0.149	0.187	10/13/06	1.51	0.836	0.100	0.084	56	45	56	45
Site #4	<38 µm	1:100	6,875	14,501	1.0219	7.026	14.818	10/13/06	1.60	8.300	0.100	0.830	12	6		
Site #4	<38 µm	1:100	6,875	14,501	1.0121	6.959	14.677	10/13/06	1.60	8.350	0.100	0.835	12	6	12	6
Site #4	<38 µm	1:1,000	6,875	14,501	0.1025	0.705	1.487	10/13/06	1.53	7.042	0.100	0.704	100	47	100	47
Site #4	<38 µm	1:10,000	6,875	14,501	0.0132	0.091	0.192	10/13/06	1.51	1.550	0.100	0.155	170	81	170	81

[a] Soil values are an average of triplicate split samples using Method 3050B.

[b] Soil XRF values.

[c] Concentrations have been corrected for filter contamination. See Table 2 and text for details.

Table 2d. Barium bioaccessibility results from Canada soils

			Barium Concentration In Soi		Mass of Soil	Mass of Barium in Soil Extracted			Post-	Barium	Volume	Mass of	Barium Bioaccessibility		Mean Barium Bioaccessibility	
Soil ID	Grain Size	Barium Ratio Solid : Fluid	3050B[a] (mg/kg)	XRF[b] (mg/kg)	Tested (g)	3050B (mg)	XRF (mg)	Extraction Date	Test pH (s.u.)	Conc. in Extract[c] (mg/L)	of Extract (L)	Barium in Extract (mg)	3050B (%)	XRF (%)	3050B (%)	XRF (%)
Site #1 Control	<2 mm	1:100	1,482	1,274	1.0085	1.495	1.285	1/17/06	1.69	2.097	0.100	0.210	14	16		
Site #1 Control	<2 mm	1:100	1,482	1,274	1.0042	1.488	1.279	1/17/06	1.69	2.063	0.100	0.206	14	16		
Site #1 Control	<2 mm	1:100	1,482	1,274	1.0017	1.485	1.276	1/17/06	1.65	2.238	0.100	0.224	15	18	14	17
Site #1 Control	<250 µm	1:100	2,201	2,063	1.0168	2.238	2.098	1/17/06	1.67	2.160	0.100	0.216	9.7	10.3		
Site #1 Control	<250 µm	1:100	2,201	2,063	1.0107	2.225	2.085	1/17/06	1.67	2.141	0.100	0.214	9.6	10.3		
Site #1 Control	<250 µm	1:100	2,201	2,063	1.0086	2.220	2.081	1/17/06	1.67	2.144	0.100	0.214	9.7	10.3	9.6	10.3
Site #1 Control	<38 µm	1:100	4,209	2,175	1.0026	4.220	2.181	1/17/06	1.69	3.128	0.100	0.313	7.4	14.3		
Site #1 Control	<38 µm	1:100	4,209	2,175	1.0014	4.215	2.178	1/17/06	1.68	3.082	0.100	0.308	7.3	14.1		
Site #1 Control	<38 µm	1:100	4,209	2,175	1.0006	4.212	2.177	1/17/06	1.70	3.164	0.100	0.316	7.5	14.5	7.4	14.3
Site #1 Control	<2 mm	1:1,000	1,482	1,274	0.1011	0.150	0.129	1/17/06	1.63	0.589	0.100	0.059	39	46		
Site #1 Control	<2 mm	1:1,000	1,482	1,274	0.1017	0.151	0.130	1/17/06	1.60	0.636	0.100	0.064	42	49		
Site #1 Control	<2 mm	1:1,000	1,482	1,274	0.1036	0.154	0.132	1/17/06	1.59	0.637	0.100	0.064	41	48	41	48
Site #1 Control	<250 µm	1:1,000	2,201	2,063	0.1050	0.231	0.217	1/17/06	1.58	0.718	0.100	0.072	31	33		
Site #1 Control	<250 µm	1:1,000	2,201	2,063	0.1014	0.223	0.209	1/17/06	1.57	0.698	0.100	0.070	31	33		
Site #1 Control	<250 µm	1:1,000	2,201	2,063	0.1035	0.228	0.214	1/17/06	1.57	0.691	0.100	0.069	30	32	31	33
Site #1 Control	<38 µm	1:1,000	4,209	2,175	0.1017	0.428	0.221	1/17/06	1.58	1.358	0.100	0.136	32	61		
Site #1 Control	<38 µm	1:1,000	4,209	2,175	0.1037	0.436	0.226	1/17/06	1.60	1.361	0.100	0.136	31	60		
Site #1 Control	<38 µm	1:1,000	4,209	2,175	0.1004	0.423	0.218	1/17/06	1.59	1.341	0.100	0.134	32	61	32	61
Site #1 Control	<2 mm	1:10,000	1,482	1,274	0.0135	0.020	0.017	1/17/06	1.62	0.206	0.100	0.021	103	120		
Site #1 Control	<2 mm	1:10,000	1,482	1,274	0.0168	0.025	0.021	1/17/06	1.59	0.222	0.100	0.022	89	104		
Site #1 Control	<2 mm	1:10,000	1,482	1,274	0.0156	0.023	0.020	1/17/06	1.58	0.161	0.100	0.016	70	81	87	102
Site #1 Control	<250 µm	1:10,000	2,201	2,063	0.0130	0.029	0.027	1/17/06	1.58	0.152	0.100	0.015	53	57		
Site #1 Control	<250 µm	1:10,000	2,201	2,063	0.0128	0.028	0.026	1/17/06	1.57	0.177	0.100	0.018	63	67		
Site #1 Control	<250 µm	1:10,000	2,201	2,063	0.0111	0.024	0.023	1/17/06	1.58	0.162	0.100	0.016	66	71	61	65
Site #1 Control	<38 µm	1:10,000	4,209	2,175	0.0104	0.044	0.023	1/17/06	1.58	0.289	0.100	0.029	66	128		
Site #1 Control	<38 µm	1:10,000	4,209	2,175	0.0105	0.044	0.023	1/17/06	1.58	0.229	0.100	0.023	52	100		
Site #1 Control	<38 µm	1:10,000	4,209	2,175	0.0101	0.043	0.022	1/17/06	1.60	0.169	0.100	0.017	40	77	53	102

[a] Soil values are an average of triplicate split samples using Method 3050B.

[b] Soil XRF values.

[c] Concentrations have been corrected for filter contamination. See Table 2 and text for details.

Appendix A

Standard Operating Procedure for Extraction Test

Standard Operating Procedure:

In Vitro Method for Determination of Arsenic Bioaccessibility for Use in Estimating Cynomolgus Monkey Bioavailability

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Attachment A – Extraction Test Checklist Sheets

1. Introduction

1.1 Synopsis

This SOP describes an *in vitro* laboratory procedure to determine a bioaccessibility value for arsenic (i.e., the fraction that would be soluble in the gastrointestinal tract) for soils and solid materials. A recommended quality assurance program to be followed when performing this extraction procedure is also provided. Once this procedure is complete, the solutions are analyzed for arsenic concentration and the mass of arsenic found in the aqueous phase, as defined by filtration at the 0.45-μm pore size, is compared to the mass introduced into the test. The fraction liberated into the aqueous phase is defined as the bioaccessible fraction of that soil. The bioaccessibility value obtained from this *in vitro* method can be correlated with relative oral bioavailability values from a Cynomolgus monkey study.

2. Procedure

2.1 Sample Preparation

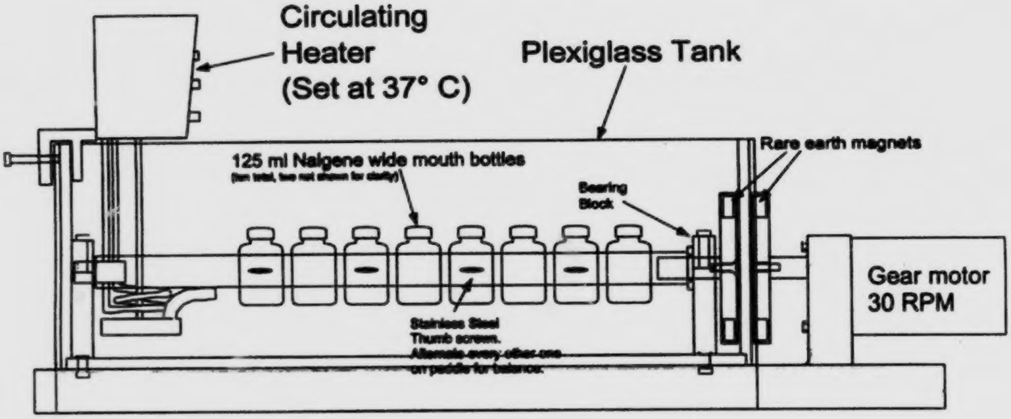
All soil/material samples should be prepared for testing by oven drying (<40 °C) and sieving to <250 μm. The <250-μm size fraction is used because this particle size is representative of that which adheres to children's hands. Subsamples for testing in this procedure should be obtained using a sample splitter.

2.2 Apparatus and Materials

2.2.1 Equipment

The main piece of equipment required for this procedure consists of a Toxicity Characteristic Leaching Procedure (TCLP) extractor motor that has been modified to drive a flywheel. This flywheel in turn drives a Plexiglass block situated inside a temperature-controlled water bath. The Plexiglass block contains ten 5-cm holes with stainless steel screw clamps, each of which is designed to hold a 125-mL wide-mouth high-density polyethylene (HDPE) bottle (see Figure 1). The water bath must be filled such that the extraction bottles are immersed. Temperature in the water bath is maintained at 37±2 °C using an immersion circulator heater (for example, Fisher Scientific Model 730). Additional equipment for this method includes typical laboratory supplies and reagents, as described in the following sections.

The 125-mL HDPE bottles must have an air-tight screw-cap seal (for example, Fisher Scientific 125-mL wide-mouth HDPE Cat. No. 02-893-5C), and care must be taken to ensure that the bottles do not leak during the extraction procedure.


Circulating
Heater
(Set at 37° C)
Plexiglass Tank
125 ml Nalgene wide mouth bottles
(ten total, two not shown for clarity)
Bearing
Block
Rare earth magnets
Gear motor
30 RPM
Stainless Steel
Thumb screws.
Alternate every other one
on paddle for balance.

Heated Tumbler
Designed by Fred Luiszer under the supervision of John Drexler
Department of Geological Sciences, University of Colorado, Boulder
Original 8-16-97 (redrawn 8-14-98)

Figure 1. Extraction device for performing the SBRC *in vitro* extraction

2.2.2 Standards and Reagents

The leaching procedure for this method uses two buffered extraction fluids at a pH of 2.5. One gram splits of each soil sample are extracted with a 0.4 M glycine and a 0.4 M Potassium Phosphate Monobasic (KH_2PO_4) solution (paired test) and the highest calculated bioaccessibility value is used to estimate the bioavailability value. The extraction fluids are prepared as described below.

The 0.4 M glycine extraction fluid should be prepared using ASTM Type II deionized (DI) water. To 1.90 L of DI water, add 60.06 g glycine (free base, Sigma Ultra or equivalent). Place the mixture in a water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using temperature compensation at 37 °C or buffers maintained at 37 °C in the water bath. Add concentrated hydrochloric acid (12.1 N, Trace Metal grade) until the solution pH reaches a value of 2.50 ±0.05 (approximately 25 mL). Bring the solution to a final volume of 2 L (0.4 M glycine).

The 0.4 M Potassium Phosphate Monobasic (KH_2PO_4) extraction fluid should be prepared using ASTM Type II deionized (DI) water. To 1.95 L of DI water, add 108.8 g KH_2PO_4 (crystalline, Fisher Certified ACS or equivalent). Place the mixture in a water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using temperature compensation at 37 °C or buffers maintained at 37 °C in the water bath. Add concentrated hydrochloric acid (12.1 N, Trace Metal grade) until the solution pH reaches a value of 2.50 ±0.05 (approximately 12 mL). Bring the solution to a final volume of 2 L (0.4 M KH_2PO_4).

Cleanliness of all reagents and equipment used to prepare and/or store the extraction fluid is essential. All glassware and equipment used to prepare standards and reagents must be properly cleaned, acid washed, and finally, rinsed with DI water prior to use. All reagents must be free of arsenic, and the final fluid should be tested to confirm that arsenic concentrations are less than 5 μg/L.

2.3 Leaching Procedure

Measure 100 ±0.5 mL of the 0.4 M glycine extraction fluid, using a graduated cylinder, and transfer into a 125-mL wide-mouth HDPE bottle. Repeat this process with a second bottle and the KH_2PO_4 extraction fluid. Add 1.00 ±0.05 g of test substrate (<250 μm) to each bottle, ensuring that static electricity does not cause soil particles to adhere to the lip or outside threads of the bottle. If necessary, use an antistatic brush to eliminate static electricity prior to adding the soil. Record the volume of solution and mass of soil added to the bottles on the extraction test checklist (see Attachment A for example checklists). Hand-tighten each bottle top, and shake/invert to ensure that no leakage occurs, and that no soil is caked on the bottom of the bottle.

Place both bottles into the modified TCLP extractor, making sure each bottle is secure and the lid(s) are tightly fastened. Fill the extractor with 125-mL bottles containing test materials or Quality Control samples.

The temperature of the water bath must be 37±2 °C. Record the temperature of the water bath at the beginning and end of each extraction batch on the appropriate extraction test checklist sheet (see Attachment A).

Rotate the extractor end over end at 30±2 rpm for 1 hour. Record start time of rotation.

When extraction (rotation) is complete, immediately remove bottles, wipe them dry, and place them upright on the bench top.

Draw extract directly from reaction vessel into a disposable 20-cc syringe with a Luer-Lok attachment. Attach a 0.45-μm cellulose acetate disk filter (25 mm diameter) to the syringe, and filter the extract into a clean 15-mL polypropylene centrifuge tube or other appropriate sample vial for analysis. Store filtered sample(s) in a refrigerator at 4 °C until they are analyzed.

Record the time that the extract is filtered (i.e., extraction is stopped). If the total elapsed time is greater than 1 hour 30 minutes, the test must be repeated.

Measure and record the pH of fluid remaining in each extraction bottle. If the fluid pH is not within ±0.5 pH units of the starting pH, the test must be discarded and the sample reanalyzed as follows.

If the pH has dropped by 0.5 or more pH units, the test will be re-run in an identical fashion. If the second test also results in a decrease in pH of greater than 0.5 s.u., the pH will be recorded, and the extract filtered for analysis. If the pH has increased by 0.5 or more units, the test must be repeated, but the extractor must be stopped at specific intervals and the pH manually adjusted down to pH 2.5 with dropwise addition of HCl (adjustments at 5, 10, 15, and 30 minutes into the extraction, and upon final removal from the water bath [60 minutes]). Samples with rising pH values must be run in a separate extraction, and must not be combined with samples being extracted by the standard method (continuous extraction).

Extracts are to be analyzed for arsenic concentration using analytical procedures taken from the U.S. EPA publication, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846 Manual*. (U.S. EPA 1999). Inductively coupled plasma-mass spectrometry (ICP-MS) analysis, method 6020 (September 1994 revision 0) will be the method of choice. This method should be adequate for determination of arsenic concentrations in sample extracts, at a project-required detection limit (PRDL) of 5 μg/L.

2.4 Calculation of the Bioaccessibility Value

A split of each solid material (<250 μm) that has been subjected to this extraction procedure should be analyzed for total arsenic concentration using analytical procedures taken from the U.S. EPA publication, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846 Manual*. (U.S. EPA 1999). The solid material should be acid digested according to method 3050B (December 1996 revision 2) or method 3051 (microwave-assisted digestion, September 1994 revision 0), and the digestate analyzed for arsenic concentration by ICP analysis (method 6010B, December 1996 revision 2). For samples that have arsenic concentrations below ICP detection limits, analysis by ICP-hydride generation (method 7061A, July 1992 revision 1) or ICP-MS (method 6020, September 1994 revision 0) will be required.

The bioaccessibility arsenic is calculated in the following manner:

$$\textit{Bioaccessibility value} = \frac{\textit{(concentration in in vitro extract, mg/L)}\ (0.1L)}{\textit{(concentration in solid, mg/kg)}\ (0.001\ kg)} \times 100$$

2.5 Chain-of-Custody/Good Laboratory Practices

All laboratories that use this SOP should receive test materials with chain-of-custody documentation. When materials are received, each laboratory will maintain and record custody of samples at all times. All laboratories that perform this procedure should follow good laboratory practices as defined in 40 CFR Part 792 to the extent practical and possible.

2.6 Data Handling and Verification

All sample and fluid preparation calculations and operations should be recorded in bound and numbered laboratory notebooks, and on extraction test checklist sheets. Each page must be dated and initialed by the person who performs any operations. Extraction and filtration times must be recorded, along with pH measurements, adjustments, and buffer preparation. Copies of the extraction test checklist sheets should accompany the data package.

3. Quality Control Procedures

3.1 Elements of Quality Assurance and Quality Control (QA/QC)

A standard method for the *in vitro* extraction of soils/solid materials, and the calculation of an associated bioaccessibility value, are specified above. Associated QC procedures to ensure production of high-quality data are as follows (see Table 1 for summary of QC procedures, frequency, and control limits):

- Reagent blanks— Both extraction fluids analyzed once per batch.
- Bottle blanks—Each extraction fluid, only run through the complete extraction procedure at a frequency of no less than 1 per 20 samples or one per extraction batch, whichever is more frequent.
- Blank spikes—Each extraction fluid spiked at 1 mg/L arsenic and run through the extraction procedure at a frequency of no less than every 20 samples or one per extraction batch, whichever is more frequent. Blank spikes should be prepared using a traceable 1,000-mg/L arsenic standard in 2 percent nitric acid.
- Duplicates—duplicate extractions are required for each extraction fluid at a frequency of 1 for every 10 samples. At least one duplicate must be performed on each day that extractions are conducted.
- Standard Reference Material (SRM)—National Institute of Standards and Technology (NIST) material 2711 (Montana Soil) should be used as a laboratory control sample (LCS) and run through the 0.4 M glycine extraction only.

Control limits for these QC samples are delineated in Table 1, and in the following discussion.

Table 1. Summary of QC samples, frequency of analysis, and control limits

QC Sample	Minimum Frequency of Analysis	Control Limits
Reagent Blanks	Once per batch per extraction fluid (min. 5%)	<5 μg/L arsenic
Bottle Blanks	Once per batch per extraction fluid (min. 5%)	<10 μg/L arsenic
Blank Spikes	Once per batch per extraction fluid (min. 5%)	85–115% recovery
Duplicates	10% for each extraction fluid	±20% RPD
SRM (NIST 2711)	2% (0.4 M glycine extraction)	To be determined

3.2 QA/QC Procedures

Specific laboratory procedures and QC steps are described in the analytical methods cited in Section 2.3, and should be followed when using this SOP.

3.2.1 Laboratory Control Sample (LCS)

The NIST SRM 2711 should be used as a laboratory control sample for the *in vitro* extraction procedure. This SRM is available from the National Institute of Standards and Technology, Standard Reference Materials Program, Room 204, Building 202, Gaithersburg, Maryland 20899 (301/975-6776).

3.2.2 Reagent Blanks/Bottle Blanks/Blank Spikes

Reagent blanks must not contain more than 5 μg/L arsenic. Bottle blanks must not contain arsenic concentrations greater than 10 μg/L. If either the reagent blank or a bottle blank exceeds these values, contamination of reagents, water, or equipment should be suspected. In this case, the laboratory must investigate possible sources of contamination and mitigate the problem before continuing with sample analysis. Blank spikes should be within 15% of their true value. If recovery of any blank spike is outside this range, possible errors in preparation, contamination, or instrument problems should be suspected. In the case of a blank spike outside specified limits, the problems must be investigated and corrected before continuing sample analysis.

4. References

U.S. EPA 1999. SW-846 Manual: Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, Updates I, II, IIA, IIB, III and IIIA (US Government Printing Office Publication Number: 955-001-00000-1). United States Environmental Protection Agency, Washington, D.C.

Attachment A:

Extraction Test Checklist Sheets

0.4 M Glycine Extraction Fluid Preparation

Date of Extraction Fluid Preparation:____________ Prepared by:_____________

Extraction Fluid Lot #:________________________

Component	Lot Number	0.4 M glycine Fluid Preparation		Acceptance Range	Actual Quantity	Comments
		1L	2L			
Deionized Water		0.95 L (approx.)	1.90 L (approx.)	---		
Glycine		30.03±0.05 g	60.06±0.05g	---		
HCl [a]		12 mL (approx.)	25 mL (approx.)	---		
Final Volume	---	1 L (Class A, vol.)	2 L (Class A, vol.)	---		
Extraction Fluid pH value (@ 37°C)	---	2.50±0.05	2.50±0.05	2.45–2.55		

[a] Concentrated hydrochloric acid (12.1 N)

0.4 M Potassium Phosphate Monobasic Extraction Fluid Preparation

Date of Extraction Fluid Preparation:____________ Prepared by:______________

Extraction Fluid Lot #:_________________________

Component	Lot Number	0.4 M KH_2PO_4 Fluid Preparation 1L	2L	Acceptance Range	Actual Quantity	Comments
Deionized Water		0.98 L (approx.)	1.95 L (approx.)	---		
Glycine		54.4±0.05 g	108.8±0.05g	---		
HCl [a]		6 mL (approx.)	12 mL (approx.)	---		
Final Volume	---	1 L (Class A, vol.)	2 L (Class A, vol.)	---		
Extraction Fluid pH value (@ 37°C)	---	2.50±0.05	2.50±0.05	2.45–2.55		

[a] Concentrated hydrochloric acid (12.1 N)

Required Parameters:

Volume of extraction fluid (V) = 100 ±0.5 mL

Mass of test substrate (M) = 1.00 ±0.05 g

Temperature of water bath = 37 ±2 °C

Extraction time = 60 ±5 min

Date of Extraction:______________________

Extraction Fluid Lot #:____________________

Extracted by:__________________________

Extractor rotation speed = 30 ±2 rpm

Maximum elapsed time from extraction to filtration = 90 minutes

Maximum pH difference from start to finish (ΔpH)= 0.5 pH units

Spike solution concentrations: As = 1 mg/L

As Spike Solution Lot #:____________________________

Extraction Log:

Sample ID	Sample Preparation		Extraction Fluid: 0.4 M Glycine _____ or 0.4 M KH_2PO_4 _____								Filtration	
	V (mL)	M (g)	Start Time[a]	End Time[a]	Elapsed Time (min)	Start pH	End pH	ΔpH	Start Temp (°C)	End Temp (°C)	Time[a]	Time Elasped from extraction (min)
Acceptance Range	(95.5–100.5)	(0.95–1.05)	---	---	(55–65)	---	---	(Max = 0.5)	(35–39)	(35–39)		(Max = 90 min)
Bottle Blank												
Matrix spike												

[a] 24-hour time scale

Analytical Procedures

QC Requirements:

QC Sample	Minimum Analysis Frequency	Control Limits	Corrective Action[a]
Reagent blanks	once per batch per extraction fluid (min. 5%)	<5 µg/L As	Investigate possible sources of arsenic. Mitigate contamination problem before continuing analysis.
Bottle blanks	once per batch per extraction solution (min. 5%)	<10 µg/L As	Investigate possible sources of arsenic. Mitigate contamination problem before continuing analysis.
Blank spikes	once per batch per extraction fluid (min. 5%)	85–115%	Re-extract and reanalyze sample batch
Duplicates	10% for each extraction fluid (min. once/day)	±20% RPD	Re-homongenize, re-extract and reanalyze

RPD – Relative percent difference
a – Action required if control limits are not met

Appendix B

Exponent Memo, June 28, 2006: Information on Early Stages of Soil Extraction Testing

Exponent

EXTERNAL MEMORANDUM

TO: Miles Tindal
FROM: Yvette Lowney
DATE: June 27, 2006
PROJECT: Barium bioaccessibility from soils BO02919.001 0301
SUBJECT: Reevaluation of Site #2 Test Soils and Rodent Information

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Bioaccessibility of Barium from Site #2 Test Soils: Barium-Free Filters

Attached are tables and figures that present data regarding the bioaccessibility of barium from the Site #2 Test Soil. The testing was conducted on three different particle sizes (<2 mm, <250 μm, and <38 μm), at three different ratios of soil to extraction fluid (1:100, 1:1,000, and 1:10,000). Additionally, all extractions were performed in triplicate to assess the potential variability across sample aliquots. The bioaccessibility data from the January and June 2006 efforts are provided in Tables 1 and 2, respectively. Table 3 provides a side-by-side comparison of the results from the different dates. Figures 1 and 2 provide a graphical representation of the results.

During Exponent's initial extraction testing effort (January 2006), the filters used to prep the extraction fluid were identified as a source of barium. As footnoted on the tables, the data were then adjusted to account for the barium introduced from the filters. The most recent extraction testing (the "June" data, reported on Table 2), was undertaken to assess whether the barium introduced into the extraction system by the filtering process had been properly accounted for in the January evaluation.

To understand the influence of filter type on the introduction of barium into the extraction system, and ideally, to identify filters that would not introduce barium into the system, five different types of filters were evaluated. The evaluation included filters from four manufacturers and two filter lots from one manufacturer. Table 4 provides a summary of the results of this assessment. These results indicate that Whatman filters (like those used for the extractions conducted in January) can be a source of barium, and that the contribution of barium was similar across different product lots. None of the other filters tested contributed appreciable levels of barium when exposed to the *in vitro* extraction fluid. Therefore, all subsequent barium extraction testing was conducted using BioExpress brand Syringe Filters from Intermountain Scientific Corporation.

The barium bioaccessibility results, as summarized in Table 3 and Figures 1 and 2, indicate that the results from the most recent extraction testing with the barium-free filters are very similar to the results reported earlier. The data show the same trend of increasing bioaccessibility with increasing soil:fluid ratio, and for extractions conducted at the two higher soil:fluid ratios, an inverse relation was observed between bioaccessibility and the soil particle size (i.e., higher bioaccessibility at the smallest particle size).

As indicated on Table 3, the bioaccessibility results from the June extractions of the 1:100 and 1:1,000 soil:fluid ratios are slightly lower than the corresponding values from January, whereas the June extractions at the 1:10,000 soil:fluid ratio are slightly higher than the values from January. These differences likely reflect random variation, especially as the values are very close, and for some samples (at the 1:10,000 extraction ratio), the range of measured values overlaps for the soil samples evaluated.

These results indicate that the extraction data provided to Axiom in January 2006 are likely accurate, having accounted for the impact of the barium-containing filters on the research results. Based on this conclusion, no further evaluations of other soils were undertaken.

Gastric Fluid Production by Rodents

Exponent also undertook a preliminary evaluation of the literature to identify information regarding gastric fluid production by rodents. Although there is a broad body of literature on acid production and the effects of drugs on pH or composition of gastric excretions in research animals including mice and rats, Exponent was unable to identify much information regarding the volume of gastric fluid production in rodents. However, one study, by Ueda et al. (1990, abstract attached), indicates that for a "control group" of rats, the animals secreted a mean 24-hour gastric juice volume of 24.4 ±2.9 mL. This information may be useful in interpreting the applicability of the bioaccessibility results from the different soil:fluid ratios.

DRAFT

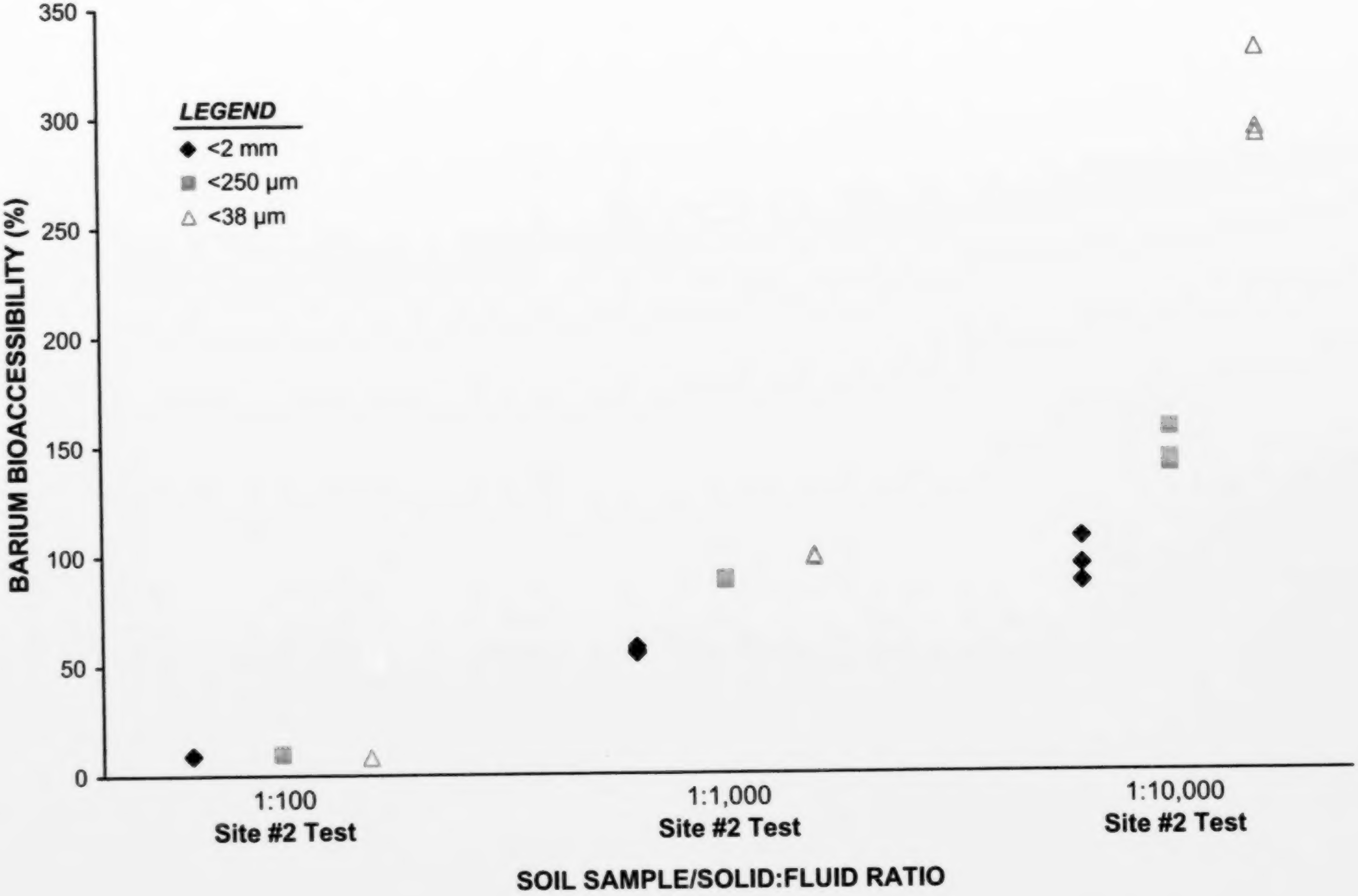

Figure 1. In vitro barium bioaccessibility of barite soils for Site #2 Test — January 2006 data

DRAFT

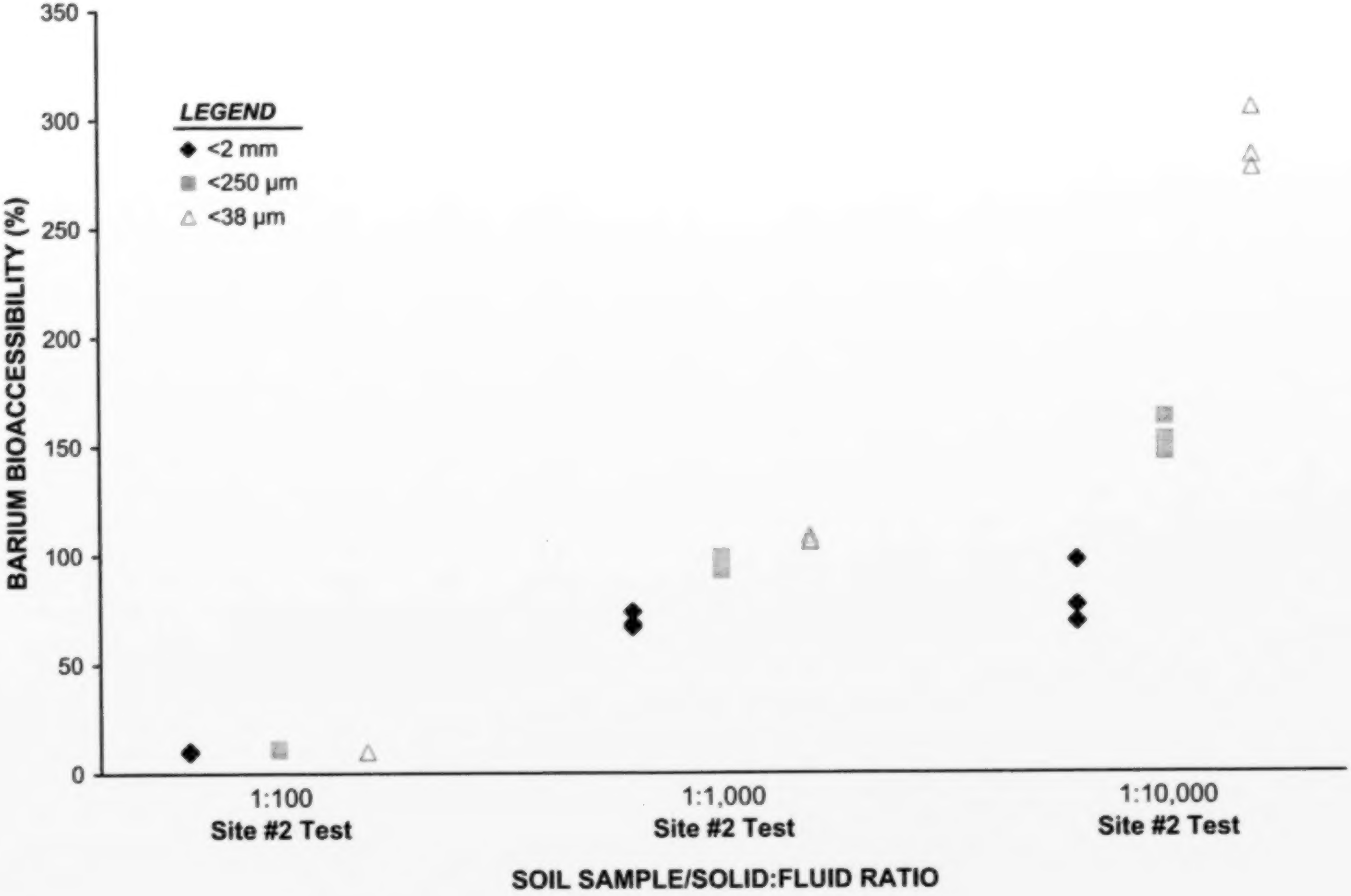

Figure 2. In vitro barium bioaccessibility of barite soils for Site #2 Test — June 2006 data

Table 1. DRAFT barium bioaccessibility results from barite soils, January 2006 data

Soil ID	Grain Size	Soil Sample Number	Bottle #	Barium Ratio Solid : Fluid	Barium Conc. in Soil[a] (mg/kg)	Mass of Soil Tested (g)	Mass of Barium in Soil Extracted (mg)	Extraction ID	Extraction Date	Post-Test pH (s.u.)	Barium Conc. in Extract[b] (mg/L)	Volume of Extract (L)	Mass of Barium in Extract (mg)	Barium Bioaccessibility (%)
Site #2 Test	<2 mm	BP19-21	19	1:100	9,712	1.0009	9.721	B063	1/25/06	1.66	8.753	0.100	0.875	9.0
Site #2 Test	<2 mm	BP19-21	19A	1:100	9,712	1.0012	9.724	B064	1/25/06	1.62	8.692	0.100	0.869	8.9
Site #2 Test	<2 mm	BP19-21	19B	1:100	9,712	1.0014	9.726	B065	1/25/06	1.62	8.561	0.100	0.856	8.8
Site #2 Test	<250 µm	BP22-24	20	1:100	8,322	1.0064	8.375	B066	1/25/06	1.64	7.834	0.100	0.783	9.4
Site #2 Test	<250 µm	BP22-24	20A	1:100	8,322	1.0018	8.337	B067	1/25/06	1.64	7.998	0.100	0.800	10
Site #2 Test	<250 µm	BP22-24	20B	1:100	8,322	1.0013	8.333	B068	1/25/06	1.65	8.001	0.100	0.800	10
Site #2 Test	<38 µm	BP25-27	21	1:100	8,737	1.0023	8.757	B069	1/25/06	1.64	7.052	0.100	0.705	8.1
Site #2 Test	<38 µm	BP25-27	21A	1:100	8,737	1.0000	8.737	B070	1/25/06	1.62	6.950	0.100	0.695	8.0
Site #2 Test	<38 µm	BP25-27	21B	1:100	8,737	1.0011	8.747	B071	1/25/06	1.64	6.901	0.100	0.690	7.9
Site #2 Test	<2 mm	BP19-21	22	1:1,000	9,712	0.1006	0.977	B073	1/25/06	1.50	5.431	0.100	0.543	56
Site #2 Test	<2 mm	BP19-21	22A	1:1,000	9,712	0.1001	0.972	B074	1/25/06	1.50	5.315	0.100	0.532	55
Site #2 Test	<2 mm	BP19-21	22B	1:1,000	9,712	0.1012	0.983	B075	1/25/06	1.53	5.649	0.100	0.565	57
Site #2 Test	<250 µm	BP22-24	23	1:1,000	8,322	0.1011	0.841	B076	1/25/06	1.51	7.425	0.100	0.743	88
Site #2 Test	<250 µm	BP22-24	23A	1:1,000	8,322	0.1007	0.838	B077	1/25/06	1.51	7.333	0.100	0.733	88
Site #2 Test	<250 µm	BP22-24	23B	1:1,000	8,322	0.1009	0.840	B078	1/25/06	1.51	7.393	0.100	0.739	88
Site #2 Test	<38 µm	BP25-27	24	1:1,000	8,737	0.1024	0.895	B079	1/25/06	1.51	8.739	0.100	0.874	98
Site #2 Test	<38 µm	BP25-27	24A	1:1,000	8,737	0.1003	0.876	B080	1/25/06	1.50	8.646	0.100	0.865	99
Site #2 Test	<38 µm	BP25-27	24B	1:1,000	8,737	0.1007	0.880	B081	1/25/06	1.51	8.689	0.100	0.869	99
Site #2 Test	<2 mm	BP19-21	25	1:10,000	9,712	0.0113	0.110	B083	1/25/06	1.58	0.948	0.100	0.095	86
Site #2 Test	<2 mm	BP19-21	25A	1:10,000	9,712	0.0126	0.122	B084	1/25/06	1.56	1.154	0.100	0.115	94
Site #2 Test	<2 mm	BP19-21	25B	1:10,000	9,712	0.0107	0.104	B085	1/25/06	1.56	1.109	0.100	0.111	107
Site #2 Test	<250 µm	BP22-24	26	1:10,000	8,322	0.0118	0.098	B086	1/25/06	1.55	1.533	0.100	0.153	156
Site #2 Test	<250 µm	BP22-24	26A	1:10,000	8,322	0.0110	0.092	B087	1/25/06	1.56	1.278	0.100	0.128	140
Site #2 Test	<250 µm	BP22-24	26B	1:10,000	8,322	0.0106	0.088	B088	1/25/06	1.54	1.253	0.100	0.125	142
Site #2 Test	<38 µm	BP25-27	27	1:10,000	8,737	0.0103	0.090	B089	1/25/06	1.53	2.959	0.100	0.296	329
Site #2 Test	<38 µm	BP25-27	27A	1:10,000	8,737	0.0107	0.093	B090	1/25/06	1.53	2.706	0.100	0.271	289
Site #2 Test	<38 µm	BP25-27	27B	1:10,000	8,737	0.0111	0.097	B091	1/25/06	1.56	2.836	0.100	0.284	292

[a] Soil values are an average of triplicate split samples.

[b] Concentrations have been corrected for filter contamination. See text for details.

Table 2. DRAFT barium bioaccessibility results from barite soils, June 2006 data

Soil ID	Grain Size	Soil Sample Number	Bottle #	Barium Ratio Solid : Fluid	Barium Conc. in Soil (mg/kg)	Mass of Soil Tested (g)	Mass of Barium in Soil Extracted (mg)	Extraction ID	Extraction Date	Post-Test pH (s.u.)	Barium Conc. in Extract (mg/L)	Volume of Extract (L)	Mass of Barium in Extract (mg)	Barium Bioaccessibility (%)
Site #2 Test	<2 mm	NA	NA	1:100	9,712	1.0015	9.727	NA	4/26/06	1.61	9.402	0.100	0.940	9.7
Site #2 Test	<2 mm	NA	NA	1:100	9,712	1.0010	9.722	NA	4/26/06	1.62	9.435	0.100	0.944	9.7
Site #2 Test	<2 mm	NA	NA	1:100	9,712	1.0010	9.721	NA	4/26/06	1.62	9.965	0.100	0.997	10.3
Site #2 Test	<250 µm	NA	NA	1:100	8,322	1.0006	8.327	NA	4/26/06	1.63	9.266	0.100	0.927	11.1
Site #2 Test	<250 µm	NA	NA	1:100	8,322	1.0007	8.327	NA	4/26/06	1.64	9.009	0.100	0.901	11
Site #2 Test	<250 µm	NA	NA	1:100	8,322	1.0009	8.329	NA	4/26/06	1.64	9.371	0.100	0.937	11
Site #2 Test	<38 µm	NA	NA	1:100	8,737	1.0002	8.739	NA	4/26/06	1.64	8.583	0.100	0.858	9.8
Site #2 Test	<38 µm	NA	NA	1:100	8,737	1.0008	8.744	NA	4/26/06	1.64	8.480	0.100	0.848	9.7
Site #2 Test	<38 µm	NA	NA	1:100	8,737	1.0000	8.737	NA	4/26/06	1.64	8.722	0.100	0.872	10.0
Site #2 Test	<2 mm	NA	NA	1:1,000	9,712	0.1003	0.974	NA	6/12/06	1.48	6.631	0.100	0.663	68
Site #2 Test	<2 mm	NA	NA	1:1,000	9,712	0.1011	0.982	NA	6/12/06	1.48	7.231	0.100	0.723	74
Site #2 Test	<2 mm	NA	NA	1:1,000	9,712	0.1004	0.975	NA	6/12/06	1.48	6.499	0.100	0.650	67
Site #2 Test	<250 µm	NA	NA	1:1,000	8,322	0.1007	0.838	NA	6/12/06	1.47	7.754	0.100	0.775	93
Site #2 Test	<250 µm	NA	NA	1:1,000	8,322	0.1007	0.838	NA	6/12/06	1.47	7.962	0.100	0.796	95
Site #2 Test	<250 µm	NA	NA	1:1,000	8,322	0.1001	0.833	NA	6/12/06	1.47	8.231	0.100	0.823	99
Site #2 Test	<38 µm	NA	NA	1:1,000	8,737	0.1008	0.881	NA	6/12/06	1.47	9.313	0.100	0.931	106
Site #2 Test	<38 µm	NA	NA	1:1,000	8,737	0.1009	0.882	NA	6/12/06	1.47	9.368	0.100	0.937	106
Site #2 Test	<38 µm	NA	NA	1:1,000	8,737	0.1003	0.876	NA	6/12/06	1.47	9.506	0.100	0.951	109
Site #2 Test	<2 mm	NA	NA	1:10,000	9,712	0.0110	0.107	NA	6/12/06	1.49	0.734	0.100	0.073	69
Site #2 Test	<2 mm	NA	NA	1:10,000	9,712	0.0107	0.104	NA	6/12/06	1.48	0.797	0.100	0.080	76
Site #2 Test	<2 mm	NA	NA	1:10,000	9,712	0.0103	0.100	NA	6/12/06	1.48	0.972	0.100	0.097	97
Site #2 Test	<250 µm	NA	NA	1:10,000	8,322	0.0102	0.085	NA	6/12/06	1.47	1.373	0.100	0.137	162
Site #2 Test	<250 µm	NA	NA	1:10,000	8,322	0.0106	0.088	NA	6/12/06	1.47	1.290	0.100	0.129	146
Site #2 Test	<250 µm	NA	NA	1:10,000	8,322	0.0101	0.084	NA	6/12/06	1.47	1.275	0.100	0.128	152
Site #2 Test	<38 µm	NA	NA	1:10,000	8,737	0.0102	0.089	NA	6/12/06	1.47	2.509	0.100	0.251	282
Site #2 Test	<38 µm	NA	NA	1:10,000	8,737	0.0102	0.089	NA	6/12/06	1.47	2.716	0.100	0.272	304
Site #2 Test	<38 µm	NA	NA	1:10,000	8,737	0.0106	0.093	NA	6/12/06	1.49	2.557	0.100	0.256	276

Table 3. Comparison of barium bioaccessibility (%)
Site #2 Test soil results for January and June, 2006

Soil:Fluid Ratio	Soil ID	Grain Size	% Barium Bioaccesibilty, January 2006 Results	% Barium Bioaccesibilty, June 2006 Results	January / June 2006 Results
1:100	Site #2 Test	<2 mm	9.0	9.7	0.93
1:100	Site #2 Test	<2 mm	8.9	9.7	0.92
1:100	Site #2 Test	<2 mm	8.8	10.3	0.86
1:100	Site #2 Test	<250 µm	9.4	11.1	0.84
1:100	Site #2 Test	<250 µm	9.6	10.8	0.89
1:100	Site #2 Test	<250 µm	9.6	11.3	0.85
1:100	Site #2 Test	<38 µm	8.1	9.8	0.82
1:100	Site #2 Test	<38 µm	8.0	9.7	0.82
1:100	Site #2 Test	<38 µm	7.9	10.0	0.79
1:1000	Site #2 Test	<2 mm	55.6	68.1	0.82
1:1000	Site #2 Test	<2 mm	54.7	73.7	0.74
1:1000	Site #2 Test	<2 mm	57.5	66.7	0.86
1:1000	Site #2 Test	<250 µm	88.3	92.5	0.95
1:1000	Site #2 Test	<250 µm	87.5	95.1	0.92
1:1000	Site #2 Test	<250 µm	88.0	98.8	0.89
1:1000	Site #2 Test	<38 µm	97.7	105.7	0.92
1:1000	Site #2 Test	<38 µm	98.7	106.2	0.93
1:1000	Site #2 Test	<38 µm	98.8	108.5	0.91
1:10,000	Site #2 Test	<2 mm	86.4	68.9	1.25
1:10,000	Site #2 Test	<2 mm	94.3	76.5	1.23
1:10,000	Site #2 Test	<2 mm	106.7	97.0	1.10
1:10,000	Site #2 Test	<250 µm	156.1	162.2	0.96
1:10,000	Site #2 Test	<250 µm	139.6	146.4	0.95
1:10,000	Site #2 Test	<250 µm	142.0	152.0	0.93
1:10,000	Site #2 Test	<38 µm	328.8	282.3	1.16
1:10,000	Site #2 Test	<38 µm	289.5	304.2	0.95
1:10,000	Site #2 Test	<38 µm	292.4	276.4	1.06

Appendix III

Extractable Barium
Analytical Method

APPENDIX III – EXTRACTABLE BARIUM ANALYTICAL METHOD

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Table VI-3. Ruggedness Testing Results for Spiked Soil Sample

1. RUGGEDNESS TESTING

This Appendix presents the results of ruggedness testing of the soil-extraction and analysis procedure for determining extractable barium. The soil used was tested with and without a spike of barium acetate added at the rate of 160 mg Ba/kg. The coefficient of variation in the data was small, 6 - 9 %. The data indicate that there were no large or consistent effects when the following factors in the procedure were systematically changed, following Youden & Steiner (1975):

- Shaking method (platform or end-over-end).
- Shaking time (2h or 16 h)
- Filtration procedure (suction or gravity).
- Delay before extract analysis (<2 h or overnight refrigeration).
- Matrix for barium standards (water or dilute Ca chloride).
- Model of ICP spectrometer (Optima or Accuris).

The ruggedness testing included eight measurements on unspiked and spiked soil. The measurements made on each soil are summarized in Table VI-1. Results of the ruggedness testing for unspiked and spiked soil are provided in Tables VI-2 and VI-3, respectively.

The extraction ratio was always 10:1. When using 10 g of soil and 100 mL of solution, slightly more barium was extracted than with only 5 g and 50 mL. The difference was too small to justify using still larger quantities, but consistent enough to justify using no less than 10 g of soil. Recovery of the 160 mg/kg spike of soluble barium in the ruggedness tests was about 40 %. This value is slightly lower than in range-finding work using the same materials, done 2 months earlier. Available barium levels, in both the control and the spiked soil (L123906), apparently fell during storage. These effects are considered unlikely to occur with soils whose main source of barium is barite.

Overall, the ruggedness testing indicated that the method is suitably rugged for the purpose required.

2. RECOMMENDED ANALYTICAL METHOD

The recommended procedure for determination of extractable barium is as follows:

- Prepare 0.1 M calcium chloride (14.7 g of pure dihydrate per litre of de-ionized water).
- Use a representative sub-sample of air-dried, <2 mm soil, weighing at least 10 g.
- Add 0.1 M calcium chloride at a 1:10 ratio, *e.g.*, 10 g soil plus 100 mL, leaving adequate head-space for agitation.
- Agitate for at least 2 h on either a platform or end-over-end shaker.
- Filter the extract either by gravity or under suction, using a filter paper fine enough to yield a clear filtrate.
- Make up standard solutions (*e.g.* of barium acetate or chloride) either in DI water or in the extracting solution.
- Analyze the filtrate and standards by ICP within 24 h.

Table III-1. Description of Measurements for Ruggedness Testing

#	*Extraction Quantities*	*Shaking Method*	*Shaking Time*	*Filtering Method*	*Delay in Extraction Analysis*	*Instrument Calibration Standards*	*Analytical Instrument*
1	10 g soil/100 mL 0.1 N $CaCl_2$	end-over-end shaker	overnight	suction	Next day	In $CaCl_2$	ICP-Optima
2	10 g soil/100 mL 0.1 N $CaCl_2$	end-over-end shaker	2 h	suction	<2 hours	In DI water	ICP-Accuris
3	10 g soil/100 mL 0.1 N $CaCl_2$	Platform shaker	overnight	gravity	Next day	In DI water	ICP-Accuris
4	10 g soil/100 mL 0.1 N $CaCl_2$	Platform shaker	2 h	gravity	<2 hours	In $CaCl_2$	ICP-Optima
5	5 g soil/50 mL 0.1 N $CaCl_2$	Platform shaker	2 h	gravity	<2 hours	In $CaCl_2$	ICP-Accuris
6	5 g soil/50 mL 0.1 N $CaCl_2$	end-over-end shaker	2 h	gravity	Next day	In DI water	ICP-Optima
7	5 g soil/50 mL 0.1 N $CaCl_2$	Platform shaker	overnight	suction	<2 hours	In DI water	ICP-Optima
8	5 g soil/50 mL 0.1 N $CaCl_2$	Platform shaker	2 h	suction	Next day	In $CaCl_2$	ICP-Accuris

Appendix IV

Fedorak and Gamez (2003) – Microbial Dissolution of Barite

INVESTIGATING THE POTENTIAL FOR MICROBIAL DISSOLUTION OF Ba^{++} FROM BARITE UNDER DIFFERENT REDOX CONDITIONS

Phillip M. Fedorak and Ryan D. Gamez
Department of Biological Sciences
University of Alberta
Edmonton, Alberta
T6G 2E9

Prepared for:
Barite Soil Guidelines
Technical Steering Committee

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EXECUTIVE SUMMARY

Barite (barium sulfate) serves as a weighting agent in oilfield drilling muds and it commonly remains onsite after drilling operations are completed. The work described in this report is part of a project that was undertaken to develop soil quality guidelines for barite. Current guidelines for barium in soil appear to be based on background levels and/or the properties of soluble barium compounds. Under typical environmental conditions, barite has a low aqueous solubility and is thought to have a low bioavailability, and accordingly, soil quality guidelines for barite are expected to be less stringent than those for soluble barium.

Information in the literature has clearly demonstrated that under sulfate-reducing conditions, microbial processes will consume sulfate and thereby increase the concentrations of dissolved Ba^{++} in cultures or environments that contain barite. However, there is little or no information on the dissolution of barite by microbial activities under other redox conditions. Thus, the objective of this study was to determine whether the concentration of dissolved Ba^{++} increased in barite-containing microcosms that were subjected to various redox conditions that promoted different microbial processes. Barite was added to microcosms containing uncontaminated (background) soil, appropriate liquid media and an electron donor (either glucose or lactate). Different sets of microcosms were incubated at room temperature under aerobic, nitrate-reducing, Mn(IV)-reducing, Fe(III)-reducing, sulfate-reducing and methanogenic conditions. The amounts of soluble Ba^{++} were determined over a 4-month incubation period.

The release of soluble Ba^{++} was observed under aerobic, Mn(IV)-reducing, Fe(III)-reducing and sulfate-reducing conditions. The findings of this short-term investigation, using microcosms with ample supplies an electron donor suggest that there is a potential for barite dissolution releasing soluble Ba^{++} in soil environments that are dominated by aerobic, Mn(IV)-reducing, Fe(III)-reducing or sulfate-reducing conditions. The rates of microbial release of soluble Ba^{++} in these types of environments would likely be much slower than observed in this study, because the supply of electron donor would be rate limiting in the environment.

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1 INTRODUCTION

Barite (barium sulfate) serves as a weighting agent in oilfield drilling muds and it commonly remains onsite after drilling operations are completed. The work described in this report is part of a project that was undertaken to develop soil quality guidelines for barite. Current guidelines for barium in soil appear to be based on background levels and/or the properties of soluble barium compounds. Under typical environmental conditions, barite has a low aqueous solubility and is thought to have a low bioavailability, and accordingly, soil quality guidelines for barite are expected to be less stringent than those for soluble barium.

The toxicity of a barium salt is highly dependent on the solubility of the salt. For example, barium sulfate is extremely insoluble and it is routinely administered orally to facilitate x-ray imaging for the detection of gastric ulcers. Barium sulfate is not absorbed from the intestinal lumen in patients that are administered this suspension (Jourdan et al. 2001). In contrast, barium ions from soluble salts are very toxic, and ingestion of aqueous barium chloride has been used as a means of suicide (Jourdan et al. 2001). The antidote for ingested soluble barium is a soluble form of sulfate, which precipitates soluble Ba^{++} as the nontoxic barium sulfate in the stomach.

Microbial dissolution of barium sulfate has been reported in the literature. This typically occurs under anaerobic conditions, with sulfate-reducing bacteria being implicated or shown to be the microorganisms responsible for causing the release of soluble Ba^{++} by reducing the sulfate moiety, thereby liberating soluble Ba^{++}. The following paragraphs summarize the published literature on the topic of release of soluble Ba^{++} from barium sulfate under different laboratory and environmental conditions.

Bolze et al. (1974) incubated lake mud under anaerobic conditions in 200 mL of medium supplemented with powdered barite and H_2 as the energy source. After 17 days of incubation at 20°C, the average concentration of dissolved Ba^{++} was 3.3 mg/L in the viable cultures, whereas the average concentration in the sterile controls was 0.5 mg/L. Another set of cultures that contained barite and ferrous sulfate was also incubated and analyzed for soluble Ba^{++}. In this set, the Ba^{++} concentration was <0.1 mg/L, indicating that the dissolution of barite was suppressed by the presence of the more soluble ferrous sulfate.

Microbially-mediated release of Ba^{++} from uranium mine tailings has been investigated because of the potential simultaneous release of radioactive $^{226}Ra^{++}$. At many uranium mining and milling sites, soluble radium is co-precipitated with $BaSO_4$ yielding a $(Ba,Ra)SO_4$ precipitate that settles as a sludge that is stored in tailings ponds. Fedorak et al. (1986) analyzed two of these co-precipitates and demonstrated that on a mass basis, the sludges were predominately $BaSO_4$.

McCready et al. (1980) produced a synthetic, $(Ba,Ra)SO_4$ precipitate in which the Ba, Ra, and S were all radioactive atoms. They added this to a pure culture of the sulfate-reducing bacterium, *Desulfovibrio vulgaris*, in medium that contained lactate as the energy source and dissolved sulfate along with the insoluble sulfate from the $(Ba,Ra)SO_4$. Over a 19-day incubation at 25°C, the concentration of soluble Ba^{++} increased at nearly a linear rate to a maximum concentration of 5.4 mg/L. This was only about 3% of the concentration expected based on the amount of sulfide released from sulfate reduction. During the growth of the *D. vulgaris*, CO_2 was produced leading to the re-precipitation of much of the Ba^{++} released from $(Ba,Ra)SO_4$ as $BaCO_3$ (McCready et al. 1980).

$(Ba,Ra)SO_4$ sludges from two Canadian uranium mines were used in laboratory studies to evaluate the release of $^{226}Ra^{++}$ and Ba^{++} by indigenous microorganisms (Fedorak et al. 1986). Prior to use in the microbiological studies, these sludges were washed free of dissolved sulfate. Microbial counts showed that the samples contained $>10^4$ sulfate-reducing bacteria/mL, and nutrient supplementation experiments demonstrated that each of the five energy sources tested (glucose, lactic, pyruvic, acetic, and glycollic acids) stimulated the microbial reduction of sulfate. In general increasing concentrations of lactate increased the amounts of Ba^{++}, and $^{226}Ra^{++}$, released from the the sludges by the end of 8- to 10-week incubation periods. In one experiment, nitrate was added as a nitrogen source for the microorganisms in one of the $(Ba,Ra)SO_4$ sludges. In the presence of nitrate, gas evolution from denitrification was evident, and the release of $^{226}Ra^{++}$ was only 3% of that in the absence of nitrate (Fedorak et al. 1986). This observation suggests that nitrate-reducing conditions inhibited the release of $^{226}Ra^{++}$. Thus, it is likely that the release of Ba^{++} would also have been inhibited under nitrate-reducing conditions, although the soluble Ba^{++} concentrations were not measured during this experiment.

Martin et al. (2003) studied the release of $^{226}Ra^{++}$ from $(Ba,Ra)SO_4$ sediments in a disposal pond at a Canadian uranium mine. They analyzed porewaters taken from various depths and three different locations in the pond. One of the locations had thick vegetative growth (*Chara* sp.) whereas the other two locations had sparse vegetative growth. Among the parameters measured were dissolved oxygen and soluble iron, manganese, barium and ^{226}Ra. The dissolved oxygen concentrations dropped sharply in the sediments and these became anaerobic within a few millimeters of the sediment-water interface. Although dissolved $^{226}Ra^{++}$ and Ba^{++} were observed in most of the porewater samples, the highest concentrations were found in the location with the thick vegetation. There was a strong linear correlation between the concentrations of the dissolved $^{226}Ra^{++}$ and Ba^{++}. At two locations, elevated concentrations of dissolved iron and manganese were observed at depths where increased concentrations of dissolved $^{226}Ra^{++}$ and Ba^{++} occurred. Plant growth would provide organic carbon for the microbial community in the sediment and the overlaying water, thereby driving processes such as Fe(III) reduction, Mn(IV) reduction and sulfate reduction. These microbial processes would play a major role in the dissolution of Fe(II), Mn(II), $^{226}Ra^{++}$ and Ba^{++}.

Scale and sludge deposits formed during oil production can contain ^{226}Ra in the form of $(Ba,Ra)SO_4$. The activities of sulfate-reducing bacteria have the potential to release $^{226}Ra^{++}$ from this naturally occurring radioactive material (NORM), in the same way they release $^{226}Ra^{++}$ from uranium tailings. Phillips et al. (2001) isolated a sulfated-reducing bacterium, designated *Desulfovibrio* sp. strain AZK1, from an oil-field brine pond and tested its ability to release $^{226}Ra^{++}$ and Ba^{++} from oil-field materials containing barite as the major form of sulfate. Cultures were supplemented with lactate and incubated at 30°C. The greatest increase in dissolved Ba^{++} occurred during the first day of incubation while the dissolved sulfate was depleted from the medium. Sulfate consumption was accompanied by sulfide formation, but the release of soluble Ba^{++} was not stoichiometric with the sulfide production. In fact, the molar ratios of dissolved Ba^{++} to sulfide production was <0.002. Although the reason for the extremely low molar ratio could not be determined, Phillips et al. (2001) concluded that none of the following mechanisms was a major contributor to the low ratio: precipitation as BaS or $BaCO_3$, adsorption by cells or reactions with reduced cell products.

Baldi et al. (1996) described transformations of barium in sewage sludges from treatment plants near Florence, Italy. The major source of barium at one of the plants was from hospital x-ray wastes. These investigators also observed lower than expected soluble Ba^{++} concentrations based on the amount of sulfide that was produced. They attributed this to the formation of $BaCO_3$ and the transient appearance of BaS. Aeration of an anaerobic sewage sludge sample caused the redox potential to increase, and brought about an inexplicable decrease in the total barium in the sludge. Baldi et al. (1996) presented several possible explanations for this loss of barium, but none was very convincing.

Sulfate reduction occurs in the contaminated groundwater of the Norman Landfill in Oklahoma (Ulrich et al. 2003). Barite was found to be an important source of sulfate for this process at intermediate aquifer depths (Ulrich et al. 2003). In general, there was an inverse relationship between the groundwater concentrations of sulfate and barium. That is, as sulfate was depleted with depth, the dissolved Ba^{++} concentrations tended to increase. Upgradient from the landfill, the concentrations of dissolved organic carbon, sulfate and barium were 2.9 mg/L, 1.2 mM (approximately 120 mg/L), and 1 μM (approximately 140 μg/L), respectively. Downgradient from the landfill and within the anoxic plume, these concentrations were 159 mg/L, 0.1 mM (approximately 10 mg/L), and 52 μM (approximately 7,100 μg/L), respectively. These measurements suggest that sulfate reduction was releasing Ba^{++} from barite in the sediments in the aquifer. Interestingly, the Fe^{++} concentration also increased, with the highest concentration being 145 times greater in the landfill leachate-contaminated groundwater than in the background sample of groundwater. The dissolved Ba^{++} and Fe^{++} were presumed to have originated from constituents of the alluvium.

Ulrich et al. (2003), also performed laboratory studies to verify that Ba^{++} release was due to the activities of sulfate-reducing bacteria. The sediment slurries incubated in the laboratory clearly demonstrated that sulfate consumption led to soluble Ba^{++} release from naturally occurring barite and from supplemented barium sulfate. Furthermore, some cultures were treated with molybdate (5 mM), which is a potent inhibitory of sulfate-reducing bacteria. No release of soluble Ba^{++} was observed in those cultures.

2 OBJECTIVES

It is has been clearly demonstrated that under sulfate-reducing conditions, microbial processes will consume sulfate and thereby increase the concentrations of dissolved Ba^{++} in cultures or environments that contain barite (e.g. McCready et al. 1980; Fedorak et al. 1984; Phillips et al. 2001; Ulrich et al. 2003). However, it was hypothesized that microbial activities would not increase the concentration of dissolved Ba^{++} from barite under other redox conditions including: aerobic, nitrate-reducing, Mn(IV)-reducing, Fe(III)-reducing and methanogenic conditions. Thus, the objective of this study was to determine whether the concentration of dissolved Ba^{++} increased in barite-containing microcosms that were subjected to these various redox conditions that promote different microbial processes.

3 MATERIALS AND METHODS

3.1 Soil samples

Two soil samples were provided by Komex International Ltd. One sample, designated as the contaminated soil, contained drilling mud that had been spilled in late 1985. The other soil sample, referred to as the background soil, contained no drilling mud. Both soils were collected from an oilfield lease in the vicinity of Pincher Creek, Alberta. The contaminated soil was collected from a stockpile of excavated material. The material was excavated as part of a process to remediate soil at the site contaminated with invert drilling mud (contaminants including weathered diesel and barium sulfate). The background sample was collected from subsoil in an uncontaminated part of the same lease site. Top soil was removed, and the sample was collected from a depth of approximately 1 m below ground surface.

Analyses done by Enviro-Test Laboratories showed that the barium concentrations in the background and the contaminated soils were 204 and 2,150 mg/kg, respectively. Enumerations of sulfate-reducing bacteria (done in our laboratory) gave numbers of 9/g and 9.3×10^3/g (wet weight) in the background and contaminated soils, respectively.

3.2 Microcosm preparation

One hundred and twelve microcosms were established for this study. They are summarized in Table 3.1, and described in more detail in the following sub-sections based on the terminal electron acceptor process that was studied.

The aerobic microcosms, in which O_2 was the terminal electron acceptor, were incubated in Erlenmeyer flasks with foam plugs to allow air exchange into and out of the flasks. All of the other microcosms were prepared using strict anaerobic techniques to ensure that O_2 was excluded. Each microcosm received 50 g of soil as the source of microorganisms. For the anaerobic microcosms, the soil was dispensed into sterile 158-mL serum bottles in an anaerobic chamber with an atmosphere of 5% H_2, 10% CO_2, balance N_2. Although H_2 serves as an electron donor (energy source) in most anaerobic environments, no attempt was made to remove the H_2 from the headspace gas of the serum-bottle microcosms.

To prepare the sterile controls, covered flasks or sealed serum bottles containing the 50-g soil sample were autoclaved for 1 h on 3 consecutive days. After adding the sterile medium to the autoclaved soil, the controls were supplemented with chloroform to a final concentration of 1% (v/v) to further reduce the chances of microbial growth (Albrechtsen et al. 1995).

Table 3.1 Summary of the microcosms established during this study.

Condition	Incubation in	No. of sterile controls	No. of viable cultures with $BaSO_4$	No. of viable cultures without $BaSO_4$
Aerobic	Shake flasks	2	5^{e}	2^{e}
Nitrate-reducing	Serum bottles	2	5^{e}	2^{e}
Mn(IV)-reducing	Serum bottles	2	5^{e}	2^{e}
Fe(III)-reducing	Serum bottles	2	5^{e}	2^{e}
Sulfate-reducing	Serum bottles	2	$5^{c} + 5^{c}$	2^{e}
Sulfate-reducing[d]	Serum bottles	2^{d}	$5^{d, e} + 5^{d,f}$	$2^{d,c} + 2^{d,f}$
Sulfate-reducing[g]	Serum bottles	2^{g}	$5^{g,c} + 5^{g,f}$	$2^{g.c} + 2^{g,.f}$
Sulfate-reducing[g,h]	Serum bottles	$2^{g,h}$	$5^{g,h,c} + 5^{g,h,f}$	0
Methanogenic-AS[a]	Serum bottles	2	5^{e}	2^{e}
Methanogenic-NAS[b]	Serum bottles	2	5^{e}	2^{e}

[a] Supplemented with anaerobic sewage sludge as a source of methanogenic community

[b] No anaerobic sewage sludge supplementation.

[c] Supplemented with 1 g lactate/L (100 mg lactate/microcosm).

[d] Contaminated soil

[e] Supplemented with 1 g glucose/L (100 mg glucose/microcosm).

[f] Without electron donor supplementation

[g] Background soil supplemented with 10 mL SRB enrichment culture.

[h] Each microcosm contains 10,000 ppm (500 mg) of barium sulfate

For each incubation condition, two sterile controls, five or ten viable microcosms supplemented with barium sulfate, and two or four microcosms without barium sulfate were prepared (Table 3.1). High purity (98%) barium sulfate (Aldrich Chemical Company, Milwaukee, WI) was used in these experiments. In most cases, 100 mg of barium sulfate was added to the microcosms (Table 3.1), which was equivalent to a soil that contained 2,000 ppm barium sulfate. In a few cases, 500 mg of barium sulfate was added to the microcosms (Table 3.1), which was equivalent to a soil that contained 10,000 ppm barium sulfate. The appropriate amount of barium sulfate was added to the soil-containing serum bottle just before the sterile culture medium was added. The microcosms were then sealed and in most cases the viable microcosms were supplemented with an electron donor.

The media used by Fedorak et al. (1997) were modified for use in these microcosms studies. In each case, sulfate was omitted from the medium so that the only source of sulfate was the sulfate in the soil or the added barium sulfate. Sulfate-free media were used to prevent re-precipitation of any Ba^{++} that may have been released in the microcosms. Each medium contained an appropriate electron acceptor, and in the case of the sulfate-reducing microcosms, barium sulfate served as the sole sulfate source. To ensure that there would be microbial activity in the viable microcosms, suitable electron donors were added (Table 3.1). In most cases, glucose was added to the microcosms. However, lactate was added to some of the sulfate-reducing microcosms. After the glucose was depleted from the medium more glucose or lactate was added to the appropriate microcosms.

All of the microcosms were incubated in the dark at room temperature (approximately 22°C). From each group of five viable microcosms with barium sulfate (column 4, Table 3.1), only three microcosms were monitored for microbial activity. The other two microcosms were established as "spares" to be monitored if one of the other three microcosms was inadvertently damaged.

3.2.1 Aerobic microcosms

Each aerobic microcosm contained 50 g of background soil in a 500-mL Erlenmeyer with 100 mL of medium (see Appendix, section A1). Nine microcosms were prepared. Five of these contained viable cultures, with 100 mg of barite added, and were supplemented with 1 mL of a 100 g glucose/mL solution for a final glucose concentration of 1 g/L. Two aerobic microcosms contained viable cultures, but no barite was added. These were also supplemented glucose. Glucose was replenished in these seven microcosms on a weekly basis.

The last two microcosms were sterile controls. Each was prepared by adding 5 mL of water to 50 g of soil for and this was autoclaved for 1 h on 3 consecutive days. Sterile medium (100 mL) and 100 mg of barite were added to each flask along with chloroform to a final concentration of 1% v/v to minimize chances of microbial growth. No glucose was added to the sterile controls.

All of the microcosms were incubated in the dark at room temperature, shaking at 200 rpm. The two sterile controls, two viable microcosms with no barite, and three of the viable microcosms with barite were monitored for soluble Ba^{++}, and glucose. At each sampling time, about 5 mL of slurry were removed from these seven microcosms using a sterile wide-mouth, 10-mL pipette. The other two viable microcosms with barite were not monitored but kept as spares in case something happened to one of the other three microcosms.

3.2.2 Nitrate-reducing microcosms

The medium composition and method of preparation of the medium used for the nitrate-reducing microcosms are given in section A.3 of the Appendix. Only the background soil was used as a source of microorganisms in these microcosms. While in the anaerobic chamber, each soil-containing serum bottle received 100 mL of nitrate-reducing medium and 1 mL of a sterile, anoxic solution that contained 100 g glucose/mL.

3.2.3 Mn(IV)-reducing microcosms

Section A.4 in the Appendix summarizes the composition and preparation of the medium used in the Mn(IV)-reducing medium. One hundred millilitres of this medium containing a slurry of MnO_2 as the source of Mn(IV) were added to each serum bottle of background soil along with 1 mL of anoxic, sterile glucose solution.

3.2.4 Fe(III)-reducing microcosms

The medium composition and method of preparation of the medium used for the Fe(III)-reducing microcosms are given in section A.4 of the Appendix. Fe(III) was added as a slurry of $Fe(OH)_3$. Only the background soil was used as a source of microorganisms in these microcosms. In the anaerobic chamber, each soil-containing serum bottle received 100 mL of Fe(III)-reducing medium and 1 mL of a sterile, anoxic glucose solution (100 g/mL).

3.2.5 Sulfate-reducing microcosms

Sulfate-reducing microcosms were established using four different conditions (Table 3.1). These were: (1) using background soil as the source of microorganisms, and adding 100 mg of barium sulfate; (2) using contaminated soil as the source of microorganisms, and adding 100 mg of barium sulfate; (3) using background soil supplemented with a mixed culture of sulfate-reducing bacteria, and adding 100 mg of barium sulfate; and (4) using background soil supplemented with a mixed culture of sulfate-reducing bacteria, and adding 500 mg of barium sulfate. In most cases, glucose was added as the electron donor. However, few sulfate-reducing bacteria use glucose and it is typically metabolized by other microorganisms leaving products that can be used by sulfate-reducing bacteria. Thus, the addition of glucose indirectly stimulates the activities of sulfate-reducing bacteria. Some microcosms were supplemented with lactate (Table 3.1) which is used directly by many sulfate-reducing bacteria.

Section A.5 in the Appendix summarizes the composition of the medium used in the sulfate-reducing microcosms. One hundred millilitres of this sulfate-free medium were added to each serum bottle containing the appropriate soil inoculum along with 1 mL of anoxic, sterile glucose or lactate solution. Sulfate in the soil and the added barium sulfate served as the electron acceptor in these microcosms.

The numbers of sulfate-reducing bacteria in each soil sample were determined using a three-tube most probable number method (section 3.4). Because of the low numbers of sulfate-reducing bacteria in the background soil (9/g wet weight), a mixed culture of sulfate-reducing bacteria was added to some of the microcosms that received the background soil to help reduce the time to obtain active sulfate reduction in the barium sulfate-containing microcosms. This mixed culture was derived from the contaminated soil as follows. After incubating for 1 month, there was abundant growth of sulfate-reducing bacteria in each 10^{-1} dilution tube of medium in the most probable number procedure. The contents of each of these three tubes were transferred to separate serum bottles that contained 100 mL of modified Butlin's medium (Fedorak et al. 1987). These three serum-bottle cultures were incubated for 6 weeks and then the cells were collected and washed to remove residual sulfate from the modified Butlin's medium, as outlined below.

The three 100-mL cultures were shaken vigorously and equal volumes, of about 150 mL, were transferred into each of two sterile centrifuge bottles that were being flushed with O_2-free nitrogen. These were centrifuged at 16,000 x *g* for 20 min, and the supernatant was removed. Then 75 mL of sterile, anoxic 0.85% saline (0.85 g NaCl in 100 mL water) was added to each centrifuge bottle, under a stream of O_2-free nitrogen, and the cells were re-suspended. Eleven milliliters of cell suspension from each bottle were transferred to an O_2-free gas-flushed serum bottle. This was sealed and autoclaved to provide heat-killed cells for the control microcosms.

The remainder of the suspension (about 130 mL) from each centrifuge bottle was transferred into a sterile, O_2-free gas-flushed, 300-mL centrifuge bottle containing sterile, anoxic sulfate-free medium for sulfate-reducing bacteria (section A.5). Nine-millilitre portions of this cell suspension were added to each of the 24 microcosms containing the background soil sample. The residual sulfate concentration and the numbers of sulfate-reducing bacteria in the remaining suspension were determined. The cell suspension contained 9 mg sulfate/L and 2.3×10^6 sulfate-reducing bacteria/mL.

3.2.6 Methanogenic microcosms

The medium composition and method of preparation of the medium used for the methanogenic microcosms are given in section A.6 of the Appendix. Only the background soil was used as a source of microorganisms in these microcosms. While in the anaerobic chamber, each soil-containing serum bottle received 100 mL of methanogenic medium and 1 mL of a sterile, anoxic solution that contained 100 g glucose/mL. It was not known whether the background soil would contain any methanogens, which are fastidious, strict anaerobes. Therefore two sets of methanogenic microcosms were established (Table 3.1). One set received only the soil, and a second set received the soil and 10 mL of anaerobic, domestic sewage sludge from the Gold Bar Waste Water Treatment Plant in Edmonton. This sludge has served as a source of methanogenic microorganisms in many previous studies in our laboratory.

3.3 Monitoring microbial activities in the microcosms

Table 3.2 summarizes the various microcosms that were established and the times at which samples will be taken for specific chemical analyses. At each sampling time, a 10-mL syringe was used to remove slurry samples from an anaerobic microcosm. Each sample was transferred to a 15-mL, plastic screw cap tube (Sarstedt, Newton NC). The samples were then stored at -20°C until sub-samples were removed after quickly thawing the samples. The samples were centrifuged at the highest speed of a bench-top clinical centrifuge (International Equipment Company, Needham, Mass) for 5 min to provide the supernatant for analyses.

3.4 Enumeration of sulfate-reducing bacteria

Sulfate-reducing bacteria were enumerated using a three-tube most probable number method with modified Butlin's medium in Kaput®-covered tubes (Fedorak et al. 1987). Each tube contained two acid-washed 2.5-cm finishing nails to help reduce the medium and to serve as an indicator of sulfate reduction. Sulfide produced by the sulfate-reducing bacteria reacted with the nail to yield black iron sulfide. Thus, those tubes of medium that contained a black nail after 30 days incubation at room temperature, were scored positive for growth of sulfate-reducing bacteria.

Table 3.2 Summary of the microcosms and the planned monitoring schedule for this project.

Microcosms	Sampling times	Analyses done
Aerobic	0, 7, 15, 30, and 60 days	Ba^{++}, glucose
Nitrate-reducing	0, 0.5, 1, 3, and 4 months	Ba^{++}, glucose, nitrate
Mn(IV)-reducing	0, 0.5, 1, 3, and 4 months	Ba^{++}, glucose, Mn(II)
Fe(III)-reducing	0, 0.5, 1, 3, and 4 months	Ba^{++}, glucose, Fe(II)
Sulfate-reducing: Background and contaminated soils	0, 0.5, 1, 2, 3, and 4 months	Ba^{++}, glucose, sulfide
Sulfate-reducing: Background soil supplemented with SRB and 500 mg $BaSO_4$	0, 0.5, 1, 2, 3, and 4 months	Ba^{++}, sulfide
Methanogenic-without sewage	0, 0.5, 1, 3, and 4 months	Ba^{++}, glucose, sulfide, methane
Methanogenic-with sewage	0, 0.5, 1, 3, and 4 months	Ba^{++}, glucose, sulfide, methane

3.5 Analytical methods

3.5.1 Glucose analysis

Glucose concentrations were determined using a colorimetric method with 3,5-dinitrosalicylic acid (DNS) (Miller 1959). A 1-mL portion of supernatant from a microcosm (or dilutions of the supernatant yielding 1 mL final volume) was mixed with 1 mL of DNS reagent and this was heated at 90°C for 5 min. After cooling, the absorbance at 540 nm was measured using a Philips Model PU 8740 UV/VIS scanning spectrophotometer.

The DNS reagent was prepared by dissolving 10 g DNS, 300 g potassium sodium tartrate and 16 g sodium hydroxide in 1 L of warm distilled water. The reagent was stored in a sealed dark glass container.

3.5.2 Nitrate analysis

A colorimetric assay was used to monitor the concentrations of nitrate in the anaerobic cultures that were incubated under nitrate-reducing conditions. The assay was modified from that reported by Cataldo et al. (1975) and is based on the principle that nitrate in the sample causes nitration of salicylic acid under acidic conditions. This causes the formation of a yellow compound that can be detected at a wavelength of 410 nm after the sample has been adjusted to pH >12.

The method was scaled-down so that only 0.1 mL of culture supernatant was needed for the analysis. To construct a calibration curve, this volume of standard nitrate solution, containing from 1 to 60 µg nitrate per 0.1 mL, was mixed with 0.4 mL salicylic acid (5% w/v) in concentrated H_2SO_4 and allowed to react at room temperature for 20 min. The reaction mixture was then placed on ice, and 9.5 mL of 2 M NaOH was added very slowly. After cooling, the absorbance of the yellow solution was measured at 410 nm. The method gave a calibration curve which was linear up to 300 mg nitrate/L.

3.5.3 Extractable Mn(II) analysis

As part of the measurement of microbial activity under Mn(IV)-reducing conditions, the microcosms were monitored for the production of Mn(II). Analyses were done using a Perkin-Elmer AAnalyst 700 Atomic Absorption Spectrophotometer (AA) equipped with a Mn lamp, measuring the absorbance at 279.5 nm. Manganese was added to microcosms as amorphous MnO_2 [Mn(IV)] (Lovley and Phillips, 1988). Although the form of manganese [Mn(II) or Mn(IV)] cannot be differentiated by AA, only Mn(II) is extracted into 0.5 M HCl (Lovley and Phillips, 1988). Thus, microcosm samples (0.1 mL of slurry) were added to 5 mL of 0.5 M HCl to extract the Mn(II). After 15 min, this was centrifuged and the supernatant was filtered (0.22 µm), diluted in $CaCl_2$ solution and then analyzed by AA to determine the amount of Mn(II) present.

Standard manganese solutions were prepared from a commercially-available manganese solution (Mn metal in 0.3 M HNO_3, 995 μg/mL). The typical calibration curve for manganese analysis was linear up to 8 mg Mn/L.

3.5.4 Soluble Fe(II) analysis

Numerous microorganisms have recently been identified which are capable of obtaining energy for growth by coupling the oxidation of organic compounds to ferric iron reduction. The most common way to measure this activity is to monitor the increase in ferrous iron [Fe(II)] over time using the ferrozine assay, as outlined by Lovley and Phillips (1986). Ferrozine, 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,3,5-triazine, reacts with divalent iron to form a stable magenta complex which is water-soluble and can be measured spectrophotometrically (Stookey, 1970). This assay is simple, quick, and reproducible, and has been used extensively by Gieg et al. (1998) and Greene et al. (1998) to monitor microbial Fe(III)-reducing activity.

Fe(II) standards for the ferrozine assay were prepared by dissolving 1.404 g $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ in 20 mL H_2SO_4 and 50 mL H_2O. The solution was brought to volume in a 100-mL volumetric flask, resulting in a 2000 mg/L stock Fe(II) solution. This stock solution was then diluted with distilled water into several standard solutions ranging from 0 to 50 mg Fe(II)/100 mL. Depending on the amount of Fe(II) to be assayed, 0.1 mL [containing 0 to 50 μg Fe(II)] or 1.0 mL [containing 0 to 500 μg Fe(II)] of these standard solutions could then be removed for reaction with ferrozine.

For the ferrozine assay, standards or culture samples (0.1 mL) were added to test tubes containing 5 mL of 0.5 M HCl. The tubes were vortexed and allowed to sit for at least 15 min to allow the dissolution of acid-soluble iron. Then, 0.1 mL of the acid solution was added to a separate test tube containing 5 mL of ferrozine (1 g/L) in 50 mM HEPES (*N*-2-hydroxy-ethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (at pH 7). The tubes were vortexed again and the absorbance at 562 nm was measured on a Philips Model PU 8740 UV/VIS scanning spectrophotometer.

Fe(II) standards were prepared as described above and 1.0 mL of each standard was analyzed by the ferrozine assay. The calibration curve obtained by plotting A_{562} vs amount of Fe(II) (0 to 500 μg) was linear.

3.5.5 Sulfate analysis

Sulfate analyses were done by ion chromatography was using a Dionex DX600 instrument with Ionpac AS9-HC (4 mm) and AG9-HC (4 mm) columns in series. The ASRS-Ultra (4 mm) suppressor was operated in the recycle mode The mobile phase was 9 mM carbonate with a flow rate of 1 mL/min. The sample loop size was 25 μL.

3.5.6 Sulfide analysis

The method of Ulrich et al. (1997) was used to measure the concentration of sulfide in the slurries in the microcosms. This is an adaptation of a similar method described by Fossing and Jørgensen (1989). Chromium reducible sulfides (including elemental sulfur and pyrite), and acid volatile sulfides (free sulfide and FeS) were released from the slurry as H_2S which was trapped in an anoxic zinc acetate solution that was prepared by adding 50 g of zinc acetate to 500 mL of freshly boiled water, and then sparging with O_2-free nitrogen while the solution cooled. One hundred millilitre portions of the solution were dispensed into 158-mL serum bottles that had been flushed with O_2-free nitrogen, and these were stoppered and sealed.

In preparation for the analysis, a small test tube was placed into a 120-mL serum bottle and the tube and bottle were flushed with O_2-free nitrogen While still flushing, 2.5 mL of anoxic zinc acetate solution were added to the test tube. Then the bottle was stoppered and sealed leaving the test tube inside. These were placed in an anaerobic chamber and weighed.

Each microcosms was shaken well, and a sample of slightly less that 2 g (approximately 1.5 mL) of slurry was removed with a syringe and this was quickly injected into the tared serum bottle, ensuring that none of the slurry was placed into the tube of zinc acetate. The wet weight of the slurry sample was recorded. Then, using a syringe, 8 mL of 1 M Cr(II)-HCl solution was added to the slurry sample. The Cr(II)-HCl solution was prepared by placing 200 g of mossy zinc in a large bottle. The zinc was covered with 0.5 M HCl and left to react until the zinc surface was clean and bright. Then the HCl was discarded, and the bottle was then flushed with O_2-free nitrogen. Separately, 133 g of $CrCl_3{\cdot}6H_2O$ was dissolved in 500 mL of 0.5 M HCl. The Cr(III) solution was dark green. The solution was reduced by reacting it with the freshly prepared zinc while being continuously flushed with O_2-free nitrogen. After about 30 min, the solution became blue indicating the formation of Cr(II).

After adding the Cr(II)-HCl solution to the slurry sample, 4 mL of 12 M HCl were added with a syringe and then the bottle was placed on a shaker at 150 rpm for 48 h. During this time, the liberated sulfide formed H_2S which was trapped in the zinc acetate solution in the test tube. The test tube was removed from the serum bottle in order to determine the amount of sulfide that was trapped as insoluble zinc sulfide. This precipitate tended to stick to the glass wall of the test tube, making it difficult to obtain a representative sample. This problem was resolved by covering the test tube with a rubber cap, adding 4 drops of 0.5 M HCl to the tube with a syringe, and vortexing the tube. This washed the precipitate off the walls, and resulted in a pH around 5.6, which was well within the range for the test kit used for sulfide analysis. After vortexing, the rubber cap was removed and the sulfide concentration was measured using a CHEMetrics sulfide analysis kit (CHEMetrics, Calverton, VA). This analysis is based on the reaction of sulfide to produce methylene blue.

3.5.7 Methane analysis

Methane was analyzed with a Hewlett Packard model 5700A GC and the packed column (Budwill et al. 1996). Methane standards were prepared in rigid containers as outlined by Nelson (1971). Samples of 0.1 mL headspace gas from microcosms were analyzed by this method. Results were expressed as percent by volume, and these were not corrected for temperature, pressure or vapor pressure of water in the closed microcosms.

3.5.8 Soluble barium analysis

Barium analyses were done using a Perkin-Elmer AAnalyst 700 Atomic Absorption Spectrophotometer equipped with both a flame and a HGA graphite furnace system. Analyses were preformed using a barium hollow cathode lamp at 553.6 nm with a slit width of 0.2 low slit height. Absorption was measured as integration of peak area and barium concentrations were measured as µg/L.

The concentration of barium in solutions was measured using the protocol recommended by the manufacturer. Using the automated AA system, 20 µL of the sample in 0.2% (v/v) high purity nitric acid (Trace metal grade, Cat. no. A5095K-212 Fisher Scientific) was dried for 20 s at 100°C followed by 45 s at 170°C, pretreated for 25 s at 1200°C and then atomized for 5 s at 2550°C in a pyrolytically coated graphite tube.

Standards were prepared using a Ba reference solution (Certified reference standard solution, Fisher Scientific). All glassware was soaked in 5% high purity nitric acid and all plasticware was soaked in 0.2% high purity nitric acid prior to use. After 4 to 5 days of soaking, these were rinsed with deionized water (MilliQ grade) and dried before use. Supernatant samples from the microcosms were filtered through 13-mm diameter, (0.45-µm pore size) nitrocellulose filters (Millipore HAWP01300). Each filter was placed in reusable Swinnex (SX0001300) casing attached to a syringe that was used to force the sample through the filter to yield a particulate-free sample for AA analysis.

During the evaluation of the barium analysis method, some samples were ashed using a method adapted from McDonald-Stephens and Taylor (1995). Briefly, samples in borosilicate test tubes were dried at 55°C under a stream of air and then ashed at 500°C to drive off any potential interfering organic material. The residual ash was dissolved in 0.2% (v/v) high purity nitric acid and the barium concentration was determined by AA.

The amount of soluble Ba^{++} in the microcosms was expressed as "released Ba (mg/kg soil)" (wet weight). The slurry samples taken after 4 months of incubation were used to determine the wet weight of soil. The slurry was centrifuged at top speed in a clinical centrifuge for 5 min. All of the supernatant was removed, and the tube with the wet soil was weighed. Then the soil was quantitatively removed from the tube, and after the tube was washed and dried it was weighed. The mass of wet soil in the original slurry was the difference between the weights. Typically, the wet weights were between 1.7 and 2.5 g.

4 RESULTS AND DISCUSSION

4.1 Evaluation of analytical methods

Most of the analytical methods used in this study have been used extensively in previous investigations in our laboratory (Fedorak et al. 1997; Gieg et al. 1998; Greene et al. 1998), therefore they were used without further evaluation. Two exceptions were the analyses for sulfide and for soluble barium. These were both evaluated and the results are presented in the following two sections.

4.1.1 Sulfide analysis

The utility of the method used to release H_2S from chromium reducible sulfides and from acid volatile sulfides is well documented (Fossing and Jørgensen 1989; Ulrich et al. 1997). Thus we simply applied the method of Ulrich et al. (1997) to liberate H_2S from the slurry samples. We focused on the ability to recover liberated H_2S based on analyses with the CHEMetrics sulfide test kit which has been used successfully in our laboratory (Eckford and Fedorak 2002). The preparation of standard sulfide solutions is quite time consuming and these solutions must be prepared fresh each time samples are analyzed. By using the CHEMetrics sulfide test kit, there is no need to prepare standard sulfide solutions because the kit provides series of colored standards against which the reacted sample is compared. Although the method is very convenient, some accuracy is lost because of the limited range of colored standards provided. For example, two of the colored standards represent 30 mg/L and 40 mg/L sulfide. If the color of the reacted sample falls between these two standards, one must estimate the concentration within that range. However, strictly quantitative sulfide analyses were not needed for this project because we simply wanted to verify that sulfate reduction was occurring in the microcosms. An increase in sulfide concentration would verify sulfate reduction.

The method for sulfide analysis depended on recovering released sulfide as zinc sulfide in a 10% (w/v) zinc acetate solution. To evaluate the method, a fresh solution of 0.25 g of $Na_2S \cdot 9H_2O$ in 10 mL of water was prepared. Then 0.1 mL of this solution was added to two test tubes each containing 9.9 mL of 10% (w/v) zinc acetate solution. The concentration of sulfide (precipitated as zinc sulfide) in each of the final solutions was calculated to be 33 mg/L. Using the CHEMetrics sulfide test kit, the concentration of sulfide was determined to be about 35 mg/L. This confirmed that the CHEMetrics method could accurately measure the sulfide concentration in a mixture of zinc acetate and zinc sulfide.

To evaluate the recovery of sulfide using the method of Ulrich et al. (1997), 2.5 mL of the zinc sulfide in zinc acetate solution (containing 33 mg sulfide/L) was added to each of two sealed serum bottles. Each serum bottle was anoxic, and contained a test tube with 2.5 mL of zinc acetate solution. After adding the Cr(II) solution and 12 M HCl, the serum bottles were placed on a shaker at 150 rpm for 48 h. If all of the sulfide in the serum bottle was recovered as zinc sulfide in the test tube, the concentration of sulfide in the test tubes would be near 33 mg/L. Results obtained with the CHEMetrics sulfide test kit, gave concentrations of about 34 and 35 mg/L in samples from the two serum bottles. These results indicated that the method used for sulfide determination was certainly adequate for this study.

4.1.2 Soluble barium analysis

Our laboratory had no previous experience doing soluble barium (Ba^{++}) analyses, thus various aspects were investigated to ensure accurate and reproducible results. The AA spectrophotometer automatically prepared dilutions of a standard Ba^{++} solution to generate calibration curves. The calibration was linear, with correlation coefficients typically >0.99.

New Swinnex filter casings (see section 3.4.8) were ordered and many samples and standards were filtered during preliminary studies (data not presented). Subsequent re-use of the filter casings suggested that residual barium remained on casings and that rigorous cleaning of the casings was required between uses. This was verified by filtering 5 mL of MilliQ water through three randomly chosen re-used filter casings and analyzing these for barium. Then 5 mL of 0.2% high purity nitric acid was filtered through the same filter casings and analyzed for barium. No barium was detected in two of the three samples of filtered MilliQ water. In the third sample of filtered MilliQ water, the concentration of barium was 1.5 µg/L. In contrast, barium was detected in all three portions of filtered nitric acid, with concentrations ranging from 8 to 15 µg/L. Thus, it was evident that simply rinsing the filter casings with MilliQ water before re-use would not adequately remove residual barium. Hence, the casings were cleaned by soaking in 0.2% high purity nitric acids for 4 to 5 days and then rinsing with copious amounts of MilliQ water.

To verify that Ba^{++} was not lost during filtration, a solution of approximately 50 µg Ba^{++}/L was prepared and analyzed in triplicate by AA. These analyses gave a mean concentration of 46±4.2 (standard deviation) µg/L. Then three portions of this solution were filtered using three nitric acid cleaned filter casings. AA analyses gave a mean of 44±1.4 µg/L. Thus, no significant amounts of Ba^{++} were lost during filtration. In addition, this experiment also demonstrated that the nitric acid cleaned filter casing did not contaminate the sample with residual barium from previous uses.

Slurry samples from the microcosms were centrifuged in a clinical centrifuge to provide supernatants for various analyses. It was postulated that this centrifugation might be adequate to remove the insoluble barium, leaving only Ba^{++} in the supernatant. Thus, sample filtration may not be required. To test this hypothesis, a slurry containing 50 g of background soil and 100 mg of barium sulfate in 100 mL of modified Butlin's medium was prepared in a serum bottle. Slurry samples were removed and centrifuged. Three portions of the supernatant were diluted 25-fold in MilliQ water and analyzed for barium. Three additional portions of the supernatant (5 mL each) were filtered using three nitric acid cleaned filter casings, and analyzed for barium after appropriate dilutions in MilliQ water. The mean concentration of barium in the samples that were centrifuged and filtered were 45±1.3 μg/L, whereas the mean concentration of barium in the samples that were only centrifuged were 72±1.6 μg/L. The higher concentration in the sample that was only centrifuged indicated that particulate barium sulfate contributed to the overall barium concentration. Therefore, centrifugation alone was not adequate to remove barium sulfate from the supernatant, and filtration was required to yield samples for Ba^{++} analysis.

The solubility product of barium sulfate is $1x10^{-10}$ (Weast 1970). Thus, at equilibrium, the concentration of Ba^{++} would be 10^{-5} M or 1.37 mg/L. The barium concentration in the 25-fold dilution of the centrifuged and filtered supernatant (described in the previous paragraph) was 45±1.3 μg/L. Thus, in the undiluted supernatant, the concentration was 1,100 μg/L or 1.1 mg/L. This value is in good agreement with the expected concentration calculated on the solubility product.

The presence of organic matter can interfere with the AA analysis of some metals (J.L. McDonald-Stephens, University of Alberta, personal communication), but the interference can be removed by ashing samples to remove the organic matter. Thus the need to ash samples for Ba^{++} analysis was evaluated. First, a 100 μg Ba^{++}/L solution was prepared and analyzed by AA (in triplicate). Three portions of this solution were put through the ashing procedure (McDonald-Stephens and Taylor 1995) and then analyzed by AA. The mean Ba^{++} concentration of the solution that was not ashed was 101±4.1 μg/L and concentration of the solution that was ashed was 102±1.2 μg/L. These results showed excellent recovery of Ba^{++} during the ashing procedure. Then, a slurry of 50 g of the contaminated soil was made in 100 mL of modified Butlin's medium. Slurry samples were removed and centrifuged yielding a supernatant that then was filtered. Three portions of supernatant were ashed analyzed by AA and two portions of supernatant were analyzed without being ashed. In each case, the portions were diluted 25-fold in MilliQ water prior to AA analysis. The mean concentration of Ba^{++} in the samples that were not ashed was 32±7 μg/L and the mean concentration of Ba^{++} in the samples that were ashed was 30±6.5 μg/L. These concentrations were essentially the same, indicating that the time-consuming ashing procedure was not required for the analyses of Ba^{++} in samples from the microcosms.

4.2 Results from microcosm studies

A variety of microcosms were established under several different redox conditions, based on the terminal electron acceptors supplied to the microcosms. In general, for a given electron donor (such as glucose), the energy available from microbial metabolism via redox processes with different electron acceptors are as follows: aerobic > nitrate-reducing > Mn(IV)-reducing > Fe(III)-reducing > sulfate-reducing > CO_2-reducing (methanogenic) conditions. The results from the microcosm studies are presenting in this order of decreasing available energy. In the figures presented in this report, the variability in the measured parameters is shown with error bars representing one standard deviation above and below the mean. In many cases, the plotted symbols are larger than the extent of the error bars.

4.2.1 Aerobic microcosms

It was hypothesized that no Ba^{++} would be released under aerobic conditions, so the aerobic microcosms were only incubated for 2 months, whereas the anaerobic microcosms were incubated for 4 months. Because aerobic metabolism of glucose, a readily utilizable growth substrate, was expected to be rapid, the microcosms were re-supplemented with glucose on five occasions over the 2-month incubation. Figure 4.1 shows the glucose concentrations in the aerobic microcosms, and these data demonstrate the rapid consumption of glucose by microorganisms in the background soil. No glucose was added to the sterile controls.

The aerobic cultures were incubated with vigorous shaking (at 200 rpm), and from previous experience, this provides ample O_2 to cultures. Therefore, the concentrations of dissolved oxygen (the electron acceptor under aerobic conditions) were not measured during this experiment.

At various times, slurry samples were removed from the microcosms, and the concentrations of soluble Ba^{++} in supernatants were measured. These values were divided by the wet weight of soil in the slurry sample taken at 2 months, and the results are shown as the "Released Ba (mg/kg soil)" in Fig. 4.2. The data show that the amounts of soluble Ba^{++} in the sterile control ranged from 4.4 to 6 mg/kg soil over the 2-month experiment. Surprisingly, the

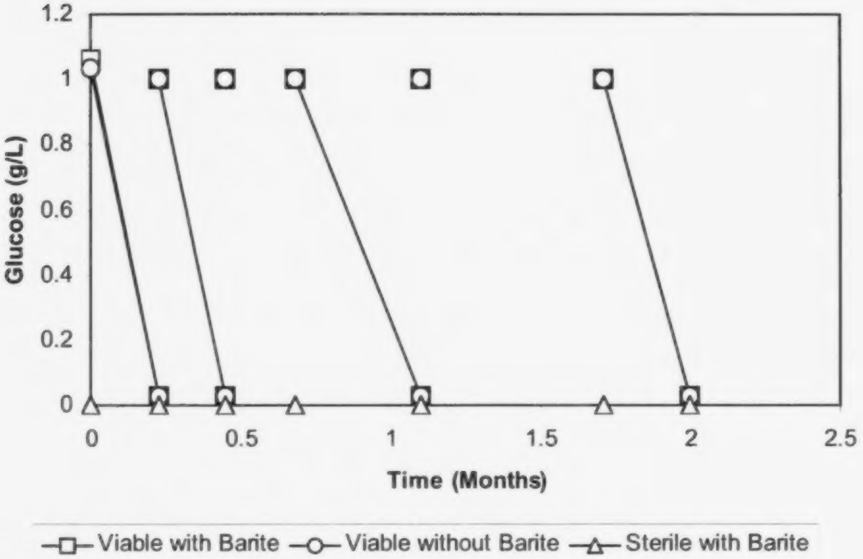

Fig. 4.1. Glucose concentrations in the aerobic microcosms. Concentrations of 1 g/L were estimated based on the amount of glucose solution added immediately after re-supplementing with at 0.2, 0.5, 0.7, 1.1, and 1.8 months. Error bars show one standard deviation.

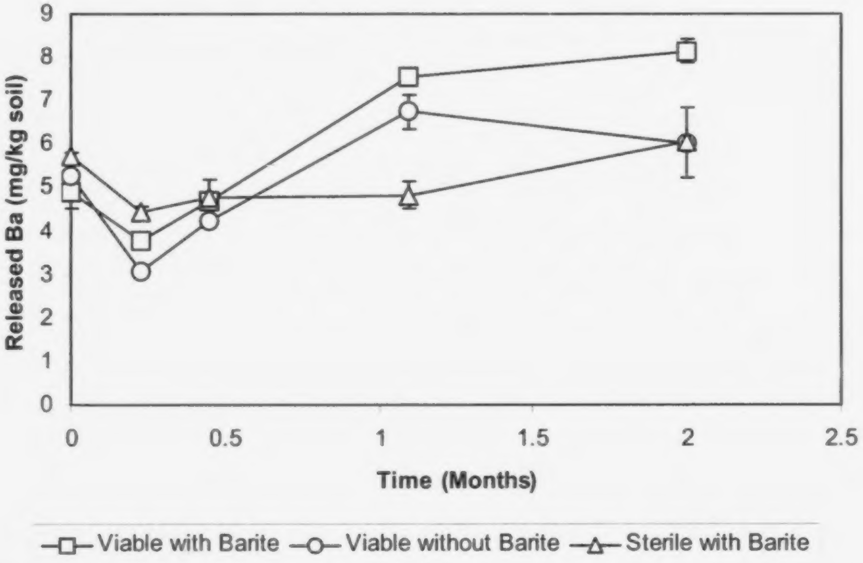

Fig. 4.2. Release of soluble Ba^{++} in the aerobic microcosms. Error bars show one standard deviation.

amounts of soluble Ba^{++} in the viable microcosms that were supplemented with 100 mg barite were higher than those in the sterile controls after 1 month and 2 months incubation (Fig. 4.2). The released Ba^{++} values were 7.5±0.2 and 8.1±0.3 mg/kg soil in the 1 month and 2 month samples, respectively. Based on t-tests, these values were statistically higher ($P<0.01$) than the corresponding control values.

The release of soluble Ba^{++} under aerobic conditions was unexpected because according to information in the literature, barite dissolution occurs under anaerobic conditions, mediated by sulfate-reducing bacteria (McCready et al. 1980; Fedorak et al. 1984; Phillips et al. 2001; Ulrich et al. 2003). However, in a nearly unintelligible report, Baldi et al. (1996) described an experiment in which barite-contaminated anaerobic sewage sludge was aerated and barium concentrations in the suspended matter were monitored. They wrote, "The experiment demonstrated that there was an 'oxidizable' fraction of barium compounds in the sewage sludge which explains the finding of lower dissolved Ba concentration in the oxidized suspended matter." This statement seems to indicate that soluble Ba^{++} was released during this 100-h experiment. We are not aware any other report of barite dissolution under aerobic conditons.

The mechanism leading to soluble Ba^{++} release in our aerobic microcosms in unknown. However, the process appears to be microbially-mediated because there was very little change in the amount of soluble Ba^{++} in the sterile controls (Fig. 4.2). The release of soluble Ba^{++} was most evident in the viable microcosms that were supplemented with barite and glucose.

4.2.2 Nitrate-reducing microcosms

Figure 4.3 summarizes the nitrate concentrations in these microcosms that contained background soil. The initial nitrate concentrations in the viable microcosms with barite and in the viable microcosms without barite were 3.1±0.0 g/L and 3.5±0.1 g/L, respectively. After 2 weeks incubation, the corresponding nitrate concentrations were 2.0±0 g/L and 2.1±0 g/L. The sterile controls (that contained 100 mg barite) were not supplemented with glucose to minimize the chance of microbial growth if they were contaminated during sampling. There was no evidence of nitrate reduction in the controls because the nitrate concentration remained at about 3.4 g/L over the 4 months of incubation the (Fig. 4.3). The nitrate concentrations in the two sets of viable cultures decreased at essentially the same rate and by month 3, the nitrate was depleted.

The initial glucose concentrations in the viable microcosms with 100 mg barite and in the viable microcosms without barite were 0.93 g/L and 0.94 g/L, respectively (Fig. 4.4). After 2 weeks of incubation, no glucose was detected in any of the viable microcosms and there was virtually no nitrate consumption between 2 weeks and 1 month of incubation (Fig. 4.3)

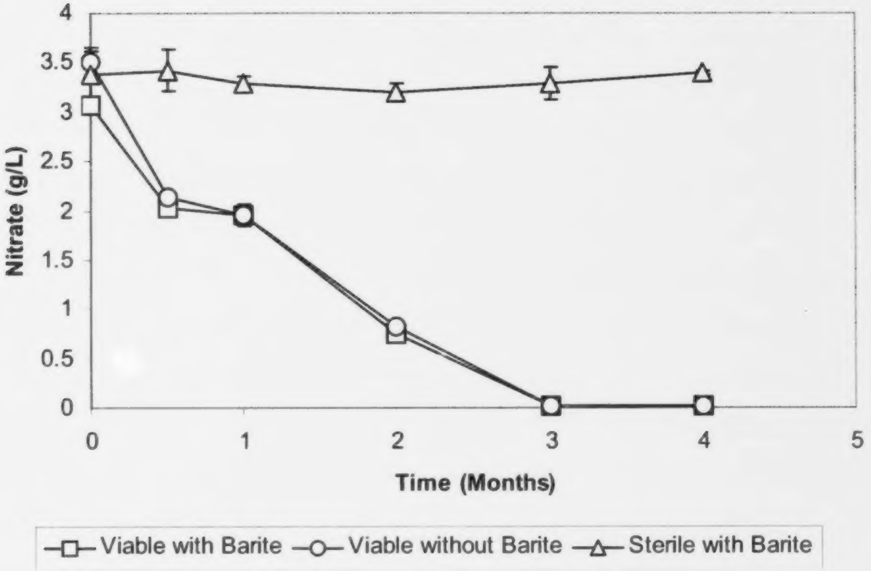

Fig. 4.3. Nitrate concentrations in the nitrate-reducing microcosms. Error bars show one standard deviation.

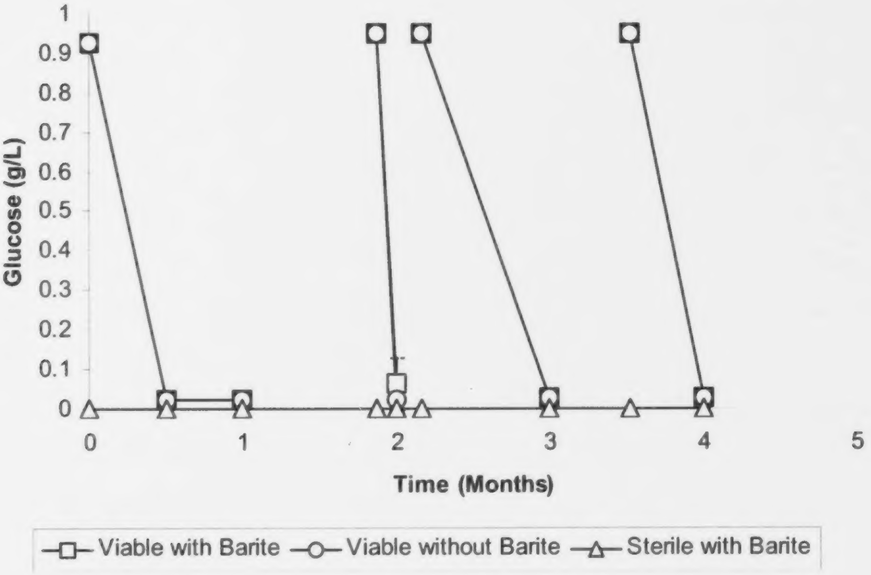

Fig. 4.4. Glucose concentrations in the nitrate-reducing microcosms. Glucose concentrations immediately after re-supplementing with glucose at 1.9, 2.2, and 3.6 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

because glucose had been depleted. To maintain the nitrate-reducing activity, the viable microcosms were re-supplemented with glucose after 57, 67, and 109 days (1.9, 2.2 and 3.6 months, respectively) of incubation (Fig. 4.4).

The results in Figs. 4.3 and 4.4 clearly indicated that the electron donor (glucose) was being consumed at the expense of the electron acceptor (nitrate). In addition, there was an increase in gas pressure in the viable microcosms, which is consistent with nitrate reduction leading to N_2 production during denitrification.

The release of soluble barium is summarized in Fig. 4.5. Surprisingly, there was an increase in the amount of soluble Ba^{++} in the "sterile" controls after 1 month of incubation. Despite the repeated autoclaving steps and the addition of chloroform to these microcosms, it appears that some microbial activity occurred in these microcosms. Figure 4.3 shows there was no consumption of nitrate in the "sterile" controls. Thus, the microbial contaminants in the "sterile" controls were likely not nitrate-reducing bacteria. The release of Ba^{++} suggests that sulfate-reducing bacteria were active in the "sterile" controls. It is often very difficult to kill all microorganisms in soil, and some genera of sulfate-reducing bacteria form endospores. These heat-resistant forms may have survived autoclaving. None of the other sterile controls prepared and monitored in this study appeared to have been contaminated with microorganisms that would release soluble Ba^{++} from barite.

Nonetheless, there was no evidence of the release of soluble barium in the viable cultures (Fig. 4.5) that consumed nitrate (Fig. 4.3) and glucose (Fig. 4.4). There is a considerable amount of information in the literature (Poduska and Anderson 1981; Jenneman et al. 1986; McInerney et al. 1996; Londry and Suflita 1999; Eckford and Fedorak 2002) showing that in the presence of nitrate, nitrate-reducing bacteria out-compete sulfate-reducing bacteria and retard sulfate reduction. Laboratory (Londry and Suflita 1999; Nemati et al 2001; Eckford and Fedorak 2002) and field (Jenneman et al. 1997) studies have demonstrated that nitrate-reducing bacteria can be stimulated in oilfield waters by the addition of nitrate. This process is used to control oilfield souring caused by microorganisms. This same mechanism likely occurred in the glucose- and nitrate-supplemented microcosms, preventing release of soluble barium in the viable cultures (Fig. 4.5) because of the stimulated activities of nitrate-reducing bacteria.

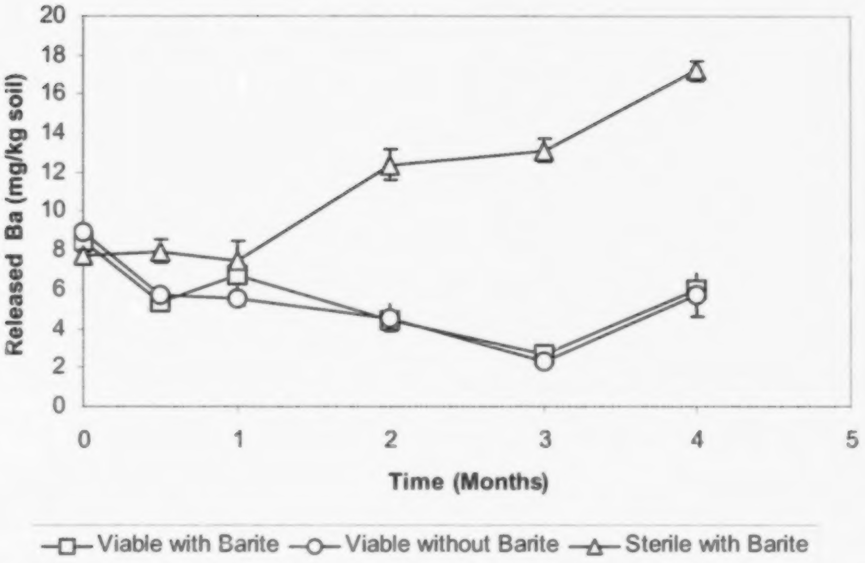

Fig. 4.5. Release of soluble Ba^{++} in the nitrate-reducing microcosms. The "sterile" controls appear to have been contaminated with sulfate-reducing bacteria. Error bars show one standard deviation.

4.2.3 Mn(IV)-reducing microcosms

Like the nitrate-reducing microcosms, the Mn(IV)-reducing microcosms contained background soil. There appeared to be no Mn(IV)-reducing activity in the sterile control, and the soluble Mn(II) concentration remained essentially constant near 0.6 g/L (Fig. 4.6). In contrast, there was an increase in the concentration of soluble Mn(II) from 0.5 to 1.9 g/L in the viable microcosms over the 4-month incubation period (Fig. 4.6). The amount of Mn(II) formed was independent of the presence of added barite in the microcosms.

Glucose was consumed rapidly in the Mn(IV)-reducing microcosms (Fig. 4.7). The initial glucose concentration in the microcosms with and without added barite were 0.95 g/L and 0.99 g/L, respectively. All the glucose was depleted after 2 weeks incubation. These microcosms were re-supplemented with glucose after 42, 52, and 93 days (1.4, 1.7, and 3.1 months, respectively) of incubation. The added glucose was consumed by the next time the microcosms were analyzed for glucose concentrations. No glucose was added to the sterile controls. The increases in the consumption of glucose and the increase in soluble Mn(II) concentrations indicate that Mn(IV) reduction occurred in the viable microcosms.

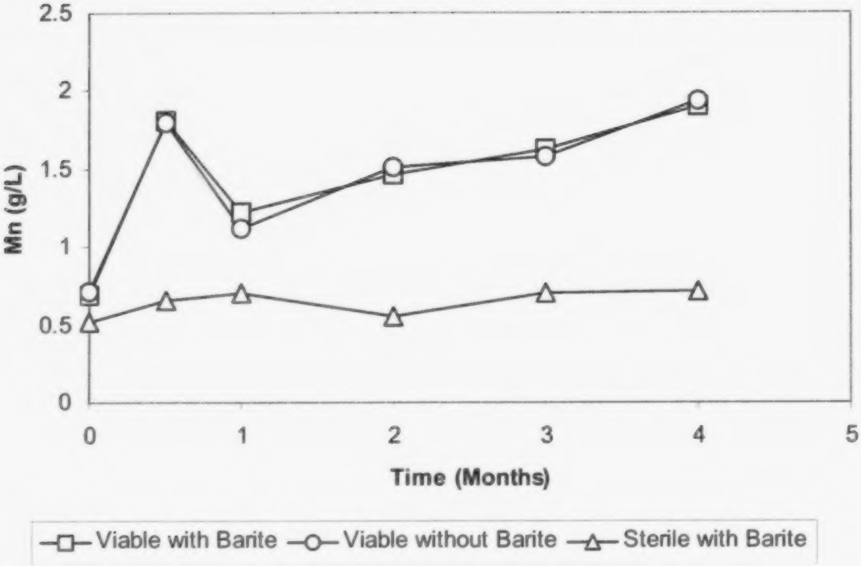

Fig. 4.6. Soluble Mn(II) concentrations in the Mn(IV)-reducing microcosm. Error bars are one standard deviation.

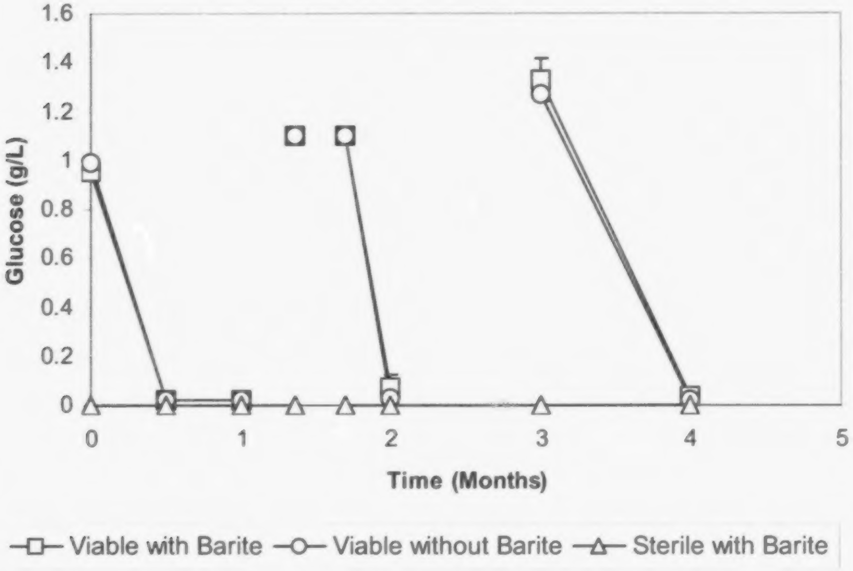

Fig. 4.7. Glucose concentrations in the Mn(IV)-reducing microcosms. Glucose concentrations immediately after re-supplementing with glucose at 1.4 and 1.7 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

Figure 4.8 shows that under Mn(IV)-reducing conditions, soluble Ba^{++} was released in the microcosms that were supplemented with barite. The rate of soluble Ba^{++} release in these microcosms was nearly linear (Fig. 4.8), and occurred concurrently with Mn(IV) reduction (Fig. 4.6).

The amount of soluble Ba^{++} increased in each of the three replicate microcosms, but the amount of release in one of these was considerably lower than in the other two replicate microcosms. This variability led to the large error bars in the 3 and 4 month averages (Fig. 4.8). However, at 4 months, the amount of soluble Ba^{++} in the viable microcosm that gave the least amount of soluble Ba^{++} release was 28 times greater than the average amount in the sterile controls.

The average amounts of soluble Ba^{++} in the barite-supplemented, viable microcosms were compared to those in the sterile controls. There was significantly more soluble Ba^{++} in the viable microcosms after 3 months of incubation ($P<0.05$), however, because of the large variability in the data, there was no statistical difference after 4 months of incubation. After 3 months of incubation, there was a 36-fold increase in the average amount of released soluble Ba^{++} in the barite-supplemented microcosms (Fig. 4.8).

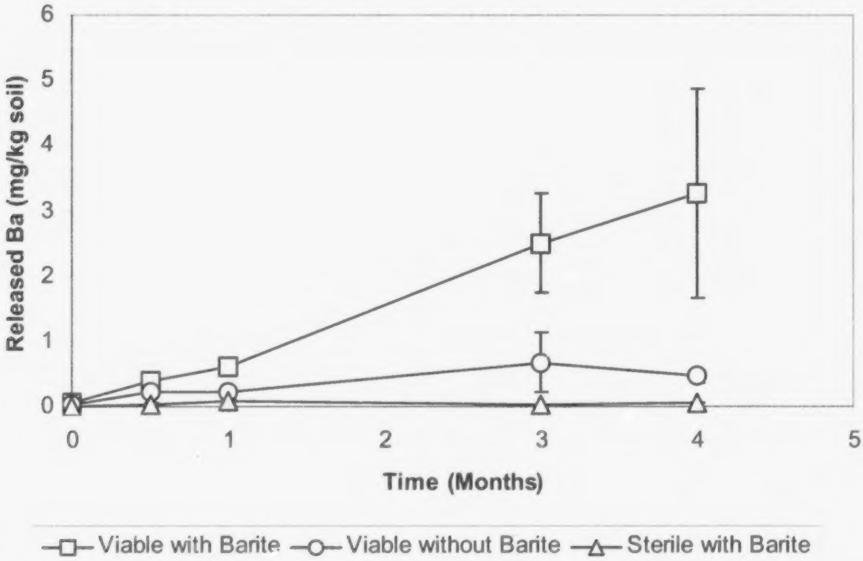

Fig. 4.8. Release of soluble Ba^{++} in the Mn(IV)-reducing microcosm. Error bars are one standard deviation.

The simultaneous release of soluble Mn(II) and Ba^{++} in these microcosms is consistent with the field observations of Martin et al. (2003) who studied the release of $^{226}Ra^{++}$ from $(Ba,Ra)SO_4$

sediments in a disposal pond at a Canadian uranium mine. They reported elevated concentrations of soluble Mn(II) and Ba^{++} at about the same depths of the sediments in the pond. Thus, it appears that Ba^{++} release can occur while active Mn(IV) reduction occurs.

4.2.4 Fe(III)-reducing microcosms

The product of Fe(III) reduction is Fe(II). The concentrations of Fe(II) increased with time in the viable microcosms that contained background soil (Fig. 4.9). The initial concentration was 0.2 g/L and after 4 months of incubation, this increased to near 4 g/L in the viable microcosms. The Fe(II) concentration in the sterile control remained constant at <0.1 g/L over this incubation time.

Glucose consumption was also observed in the Fe(III)-reducing microcosms (Fig. 4.10). Initially the microcosms with and without added barite contained glucose concentrations of 1.1 g/L. No glucose was detected after 2 weeks of incubation. The microcosms were re-supplemented with glucose after 41, 51, and 92 days (1.4, 1.7, and 3.1 months, respectively) of incubation. The consumption of glucose and the formation of Fe(II) indicated that microbial Fe(III) reduction occurred in the viable microcosms.

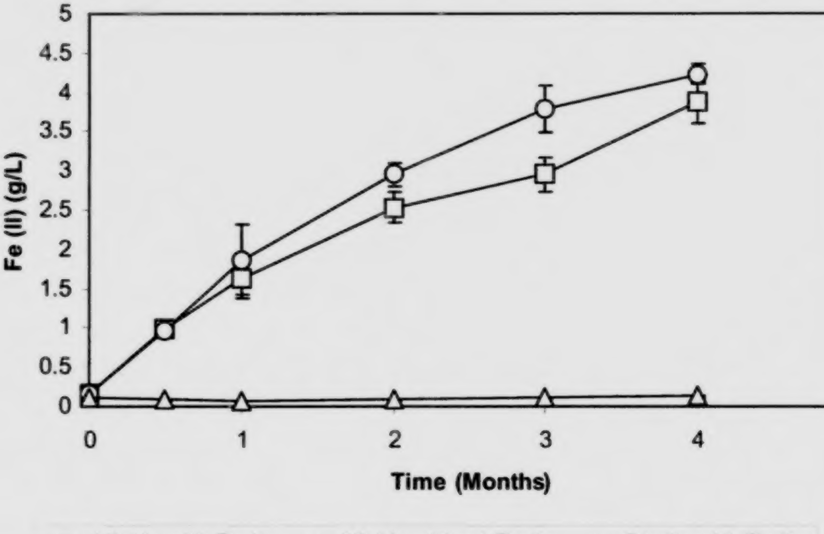

Fig. 4.9. Soluble Fe(II) concentrations in the Fe(III)-reducing microcosms. Error bars show one standard deviation.

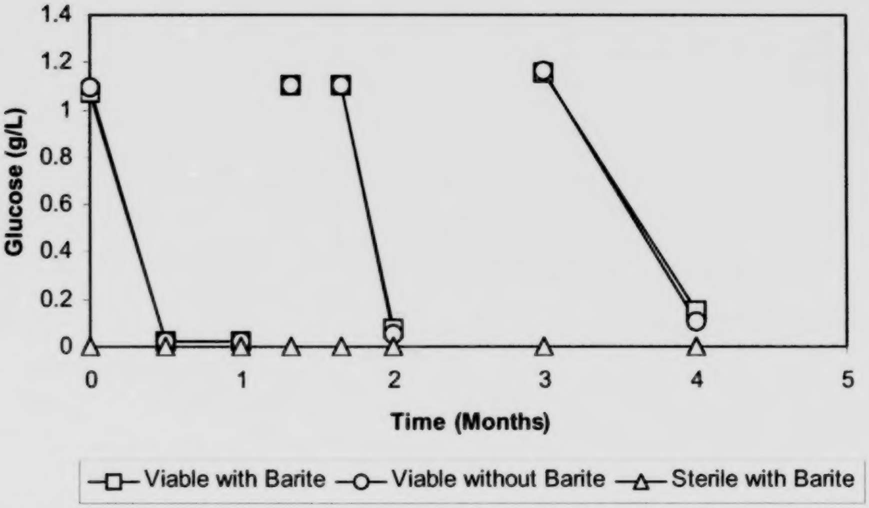

Fig. 4.10. Glucose concentrations in the Fe(III)-reducing microcosms. Glucose concentrations immediately after re-supplementing with glucose at 1.4 and 1.7 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

The release of soluble Ba^{++} was observed in microcosms that were incubated with and without added barite (Fig. 4.11). Over the 4-month incubation period, the amount of soluble Ba^{++} remained essentially constant in the sterile control, but increased 10-fold in the viable microcosms without added barite, and 43-fold in the viable microcosms with added barite. Based on t-tests, the amounts of soluble Ba^{++} in the viable microcosms were significantly greater ($P<0.01$) than in the sterile controls after 4 months incubation.

The results in Fig. 4.11 suggested that sulfate reduction might have occurred in the Fe(III)-reducing microcosms. Thus, sulfide analyses were done on the samples that were removed from these microcosm after 3 months of incubation. The sterile controls contained 3.1±0.9 µg sulfide/g soil, whereas the viable culture that was supplemented with barite contained 12±3 µg sulfide/g soil. These results indicate that sulfate reduction occurred in the Fe(III)-reducing microcosms. Fig. 4.11 shows that concentration of soluble Fe(II) increased between 1 and 3 months of incubation, thus the supply of added Fe(III) could not have been depleted during this time. This implies that both sulfate reduction and Fe(III) reduction occurred simultaneously in these microcosms.

In their studies of the release of $^{226}Ra^{++}$ from $(Ba,Ra)SO_4$ sediments in a disposal pond at a Canadian uranium mine, Martin et al. (2003) observed elevated concentrations of dissolved iron

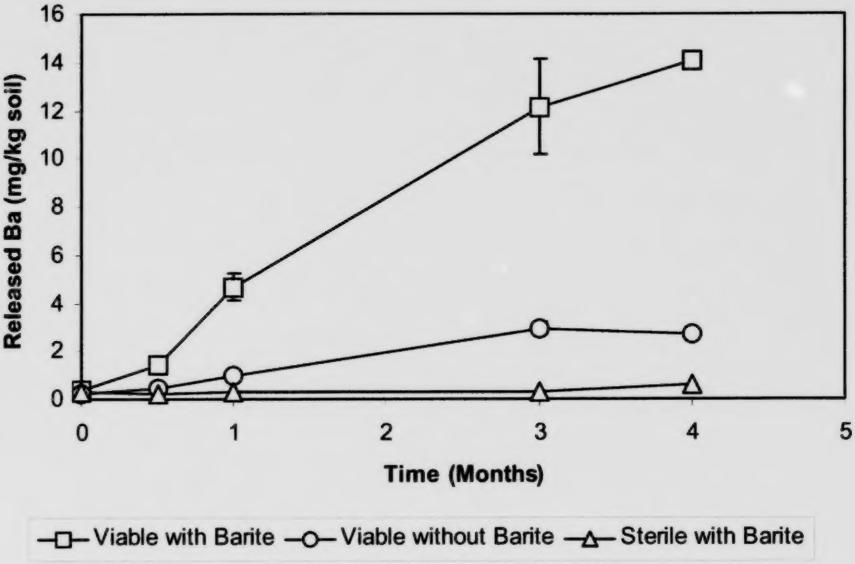

Fig. 4.11. Release of soluble Ba^{++} in the Fe(III)-reducing microcosms. Error bars show one standard deviation.

at depths where there were increased concentrations of dissolved $^{226}Ra^{++}$ and Ba^{++}. Similarly, Ulrich et al. (2003) reported an increased soluble Fe(II) and Ba^{++} concentrations downgradient from the Norman Landfill in Oklahoma. These field results suggest that Fe(III) reduction and sulfate reduction can occur in the same vicinities. Our microcosm studies show that they can occur in the same soil sample if it contains Fe(III) and sulfate to serve as terminal electron acceptors. We speculate that in the microcosms, Fe(III) reduction and sulfate reduction may be the result of different microbial populations co-existing in the soil. However, Coleman et al. (1993) demonstrated that under conditions in which the electron donor H_2 was abundant, the sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, simultaneously reduced Fe(III) and sulfate.

4.2.5 Sulfate-reducing microcosms

Several reports in the literature have demonstrated that soluble Ba^{++} is released from barite under sulfate-reducing conditions (McCready et al. 1980; Fedorak et al. 1984; Phillips et al. 2001; Ulrich et al. 2003). Thus, a variety of microcosms were established under sulfate-reducing conditions to serve as positive controls. That is, to ensure that soluble Ba^{++} release could be detected using our experimental protocols. Although the results in some of the previous sections have demonstrated that barite dissolution occurs under sulfate-reducing conditions, the results from the sulfate-reducing microcosms are presented below for completeness.

Preliminary work with the two soil samples showed that the number of sulfate-reducing bacteria in the background soil was very low (9/g wet weight). Thus, sulfate-reducing bacteria were enriched from the contaminated soil, and these were added to 24 microcosms that contained background soil. In addition, microcosms that were not amended with the enrichment culture of sulfate-reducing bacteria were also established. These contained either background soil or contaminated soil. The results from the two sets of microcosms (with or without added sulfate-reducing bacteria) are presented separately in the following two sub-sections.

4.2.5.1 Microcosms containing native sulfate-reducing bacteria

Sulfide production was observed in microcosms that contained background soil (Fig. 4.12) or contaminated soil (Fig. 4.13). Over the 4-month incubation, there was little change in the sulfide found in the sterile controls, and the addition of glucose stimulated sulfide production in both soils. The microbial community in the contaminated soil released much more sulfide than did the community in the uncontaminated soil. The concentration of sulfide in the contaminated soil slurry increased from 19 to 71 μg/g in the glucose supplemented microcosms (Fig. 4.13). In contrast, the concentration of sulfide in the background soil slurry increased from 2 to 9.6 μg/g in the glucose supplemented microcosms (Fig. 4.12). These results demonstrated that both soils had active, native sulfate-reducing bacteria.

Glucose was consumed quickly in the microcosms that contained either background soil (Fig. 4.14) or contaminated soil (Fig. 4.15). From initial concentrations of near 1 g/L, no glucose was detected after 2 weeks of incubation. All of these microcosms were re-supplemented with glucose after 25, 35, and 77 days (0.7, 1.2, and 2.6 months, respectively) of incubation.

Some of these microcosms were supplemented with lactate, rather than glucose. However, lactate analyses were not done on the supernatants from the microcosms. Nonetheless, these microcosms were re-supplemented with lactate after 35 and 77 days of incubation.

The microcosms with the viable native sulfate-reducing bacteria released soluble Ba^{++} from barite. The amount of Ba^{++} released from the glucose- and barite-supplemented microcosms containing uncontaminated soil peaked at 13 mg/kg soil (wet weight) after 2 months (Fig. 4.16) this decreased to 7.8 mg/kg soil at the end of the 4-month incubation period. Based on t-tests, both of these values were greater than those found in the controls incubated for the same time (*P*<0.01).

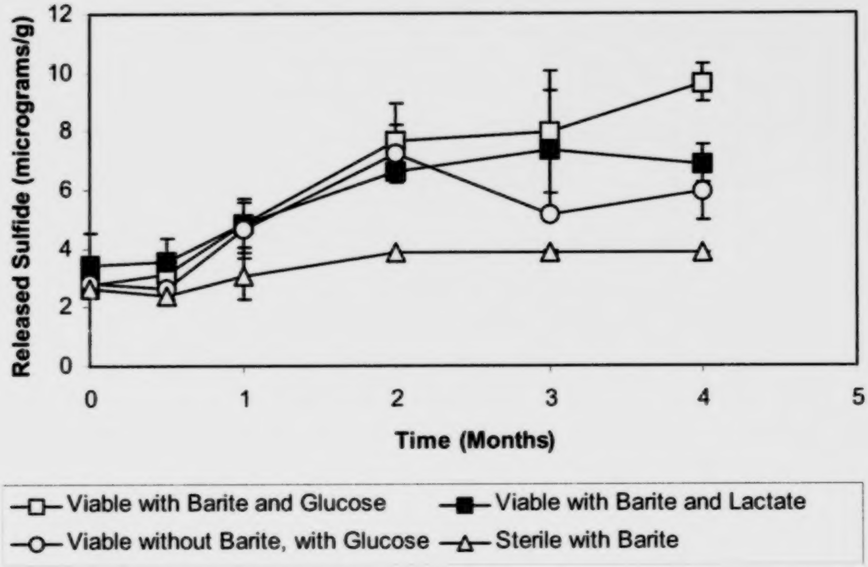

Fig. 4.12. Sulfide production in sulfate-reducing microcosms containing background soil and native sulfate-reducing bacteria. Error bars are one standard deviation.

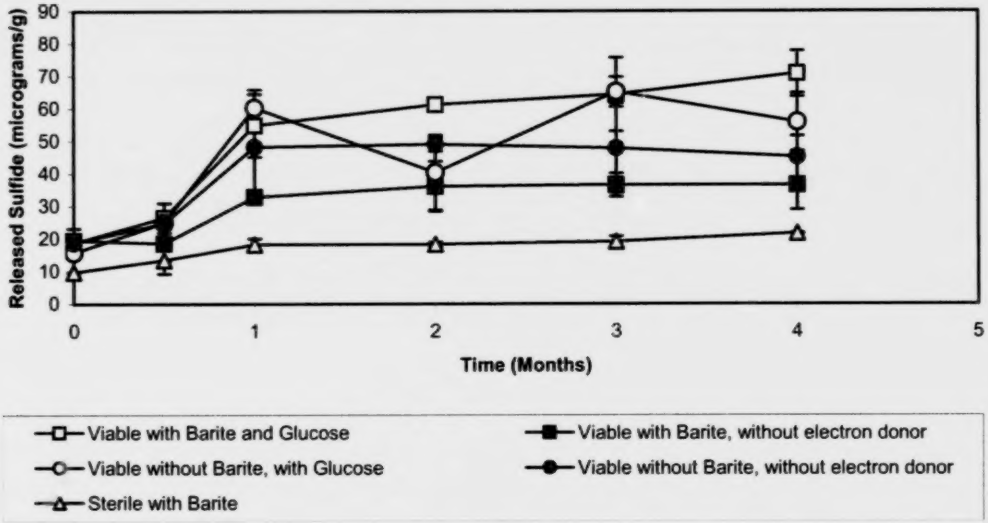

Fig. 4.13. Sulfide production in sulfate-reducing microcosms containing contaminated soil and native sulfate-reducing bacteria. Error bars are one standard deviation.

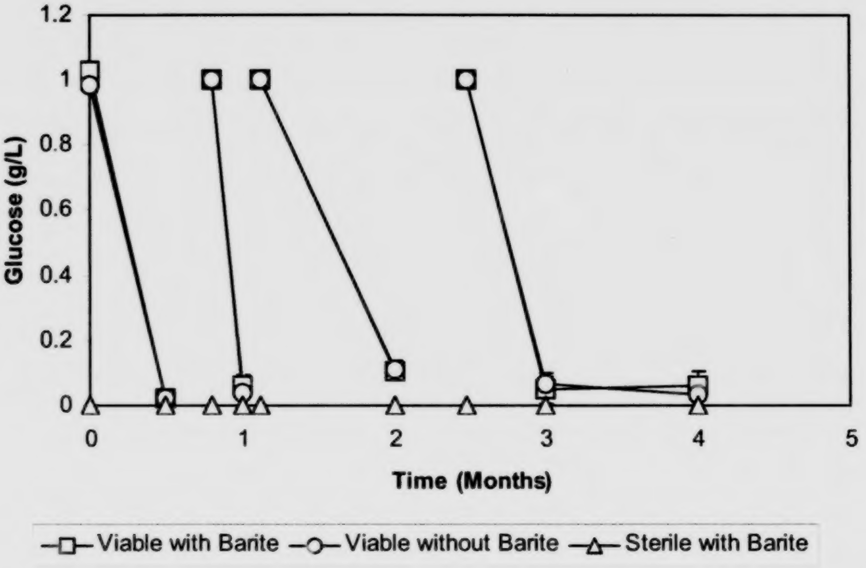

Fig. 4.14. Glucose concentrations in the sulfate-reducing microcosms containing background soil and native sulfate-reducing bacteria. Glucose concentrations immediately after re-supplementing with glucose at 0.7, 1.2, and 2.6 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

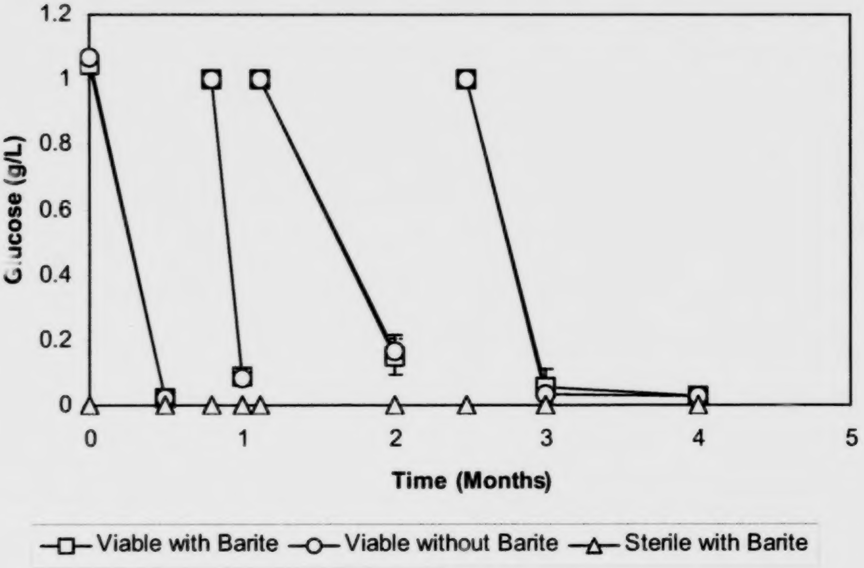

Fig. 4.15. Glucose concentrations in the sulfate-reducing microcosms containing contaminated soil and native sulfate-reducing bacteria. Glucose concentrations immediately after re-supplementing with glucose at 0.7, 1.2, and 2.6 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

Lactate is commonly used by many sulfate-reducing bacteria. Surprisingly, the addition of lactate to the background soil did not stimulate Ba^{++} release as quickly as did the addition of glucose (Fig. 4.16). However, the production of sulfide was observed in these microcosms (Fig. 4.12).

When supplemented with glucose, the microbial community in the contaminated soil released soluble Ba^{++} from barite (Fig. 4.17). The contaminated soil contained barite from drilling mud, and by the end of the 4-month incubation, the amount of soluble Ba^{++} released in viable microcosms with or without added barite were essentially the same (13 mg/kg soil). This value was significantly ($P<0.05$) higher than those in the viable microcosms without glucose (3.7 mg/kg soil) and in the sterile controls (0.7 mg/kg soil).

The barite-contaminated soil also had a much higher count of sulfate-reducing bacteria (9.3×10^3/g wet weight) than the background soil (9/g wet weight). The initial sulfide content was also higher in the contaminated soil-containing microcosms than in the background soil-containing microcosms. These concentrations were 19 µg/g (Fig. 4.13) and 3 µg/g (Fig. 4.12), respectively. The higher number of sulfate-reducing bacteria and the higher initial sulfide concentrations are consistent with active sulfate reduction occurring *in situ* in the contaminated soil. However, the initial soluble Ba^{++} concentrations in the time zero samples from the microcosms that contained contaminated soil were essentially the same as those from the microcosms that contained background soil. These concentrations were near 3 mg/kg soil (Figs. 4.16 and 4.17).

4.2.5.2 Microcosms supplemented with an enrichment culture of sulfate-reducing bacteria

Because of the low numbers of sulfate reducers in the background soil, 24 microcosms were supplemented with 9 mL of a cell suspension that contained 2.3×10^6 sulfate-reducing bacteria/mL. After supplementation, the number sulfate reducers was equivalent to 4.1×10^5/g (wet weight) of soil. The enrichment culture was grown with lactate as the major carbon and energy source, so lactate, rather than glucose, was added to the microcosms as the electron donor. These microcosms were re-supplemented with lactate after 28 and 70 days of incubation.

Two sets of microcosms were established to determine if the concentration of barite influenced the microbial activities. One set of microcosms was supplemented with 2000 ppm barite (the amount used in all of the previously discussed microcosms), and the second set was

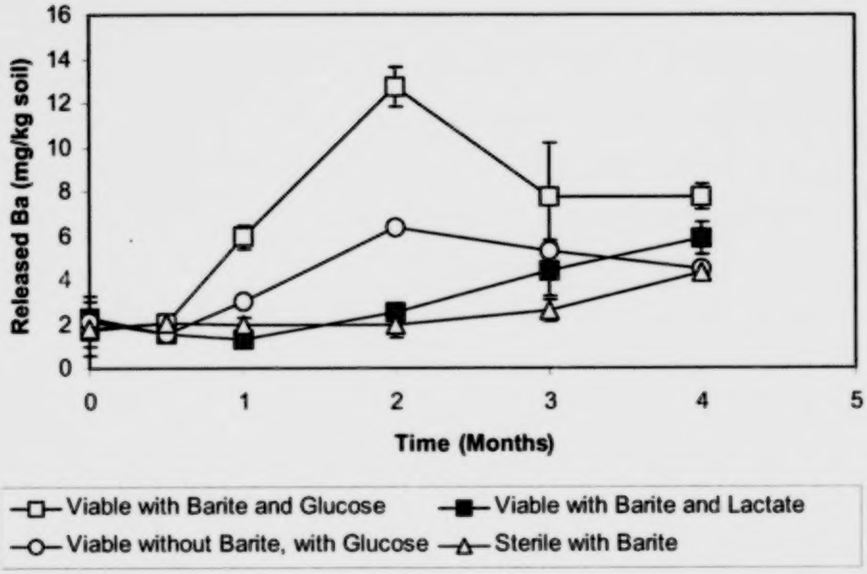

Fig. 4.16. Release of soluble Ba^{++} in the sulfate-reducing microcosms containing background soil and native sulfate-reducing bacteria. Error bars show one standard deviation.

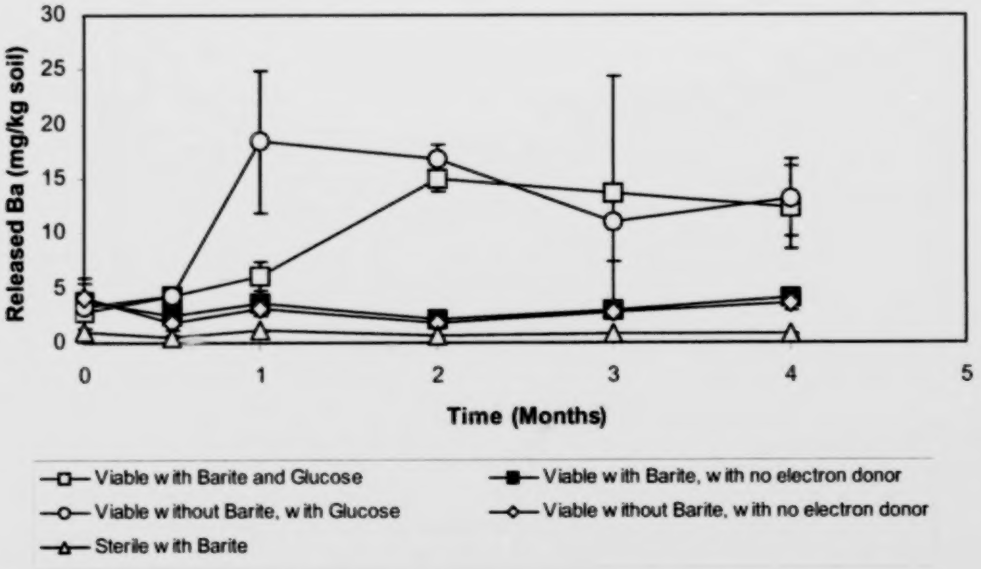

Fig. 4.17. Release of soluble Ba^{++} in the sulfate-reducing microcosms containing contaminated soil and native sulfate-reducing bacteria. Error bars show one standard deviation.

supplemented with 10,000 ppm barite. Sulfide production and release of soluble Ba^{++} were observed in both sets of microcosms. Figs. 4.18 and 4.19 summarizes the sulfide production in these microcosms.

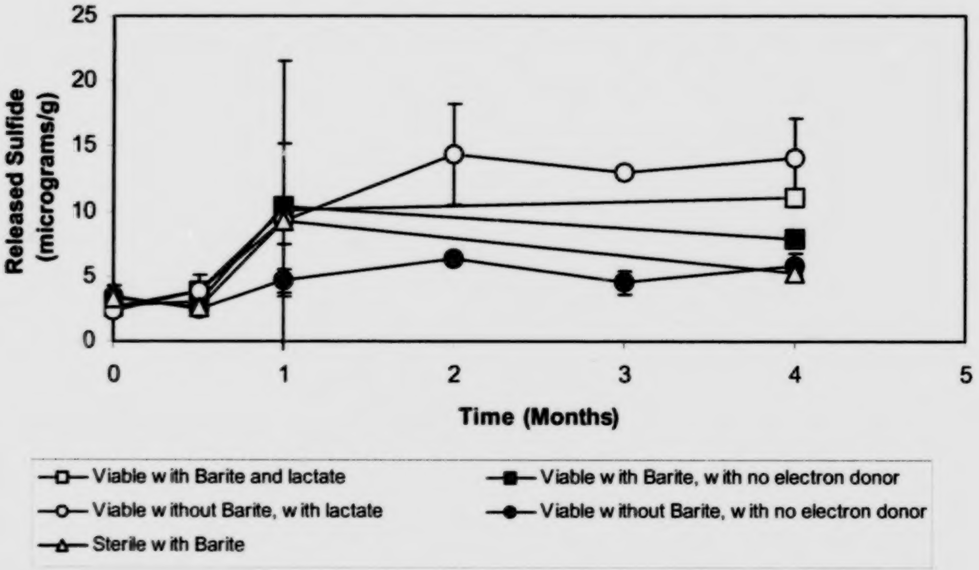

Fig. 4.18. Sulfide production in sulfate-reducing microcosms containing background soil supplemented with sulfate-reducing bacteria and 2000 ppm barite. Error bars are one standard deviation.

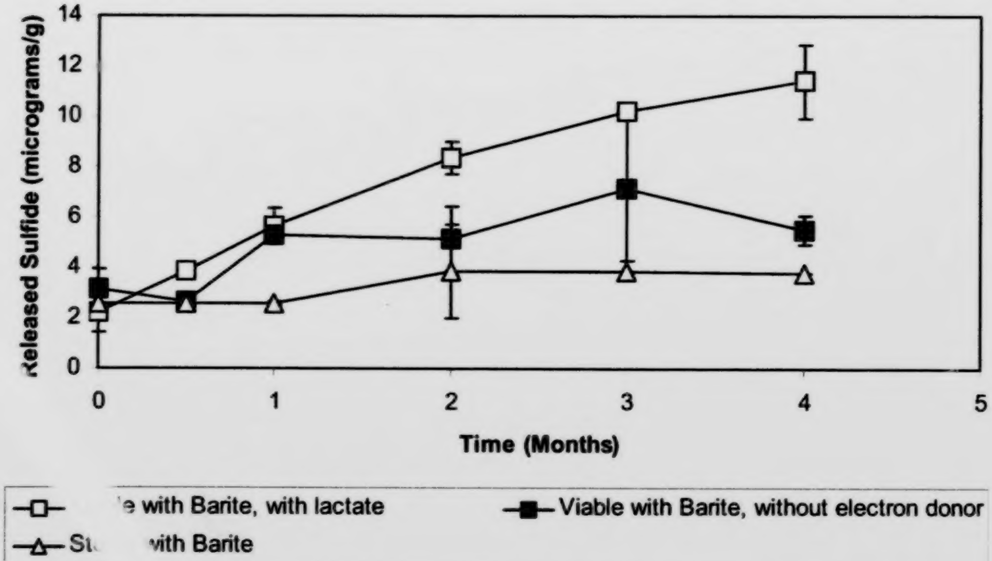

Fig. 4.19. Su[illegible] production in sulfate-reducing microcosms containing background soil supplemented with sulfate-reducing bacteria and 10,000 ppm barite. Error bars are one standard deviation.

Considering the data after 4 months incubation, the sulfide concentration in the viable culture supplemented with lactate and 2000 ppm barite was significantly greater than that in the sterile control ($P<0.01$). Fig. 4.18 shows these concentrations to be 11±0.7 and 5.2±0.1 μg/g soil, respectively. Similarly after 4 months incubation, the sulfide concentration in the viable culture supplemented with lactate and 10,000 ppm barite was significantly greater than that in the sterile control ($P<0.01$). Figure 4.19 shows these concentrations were 11±1.5 and 3.2±0.01 μg/g soil, respectively. The different amounts of barite added to the microcosms did not affect the final sulfide concentrations in these microcosms. After 4 months incubation, those that contained 2000 ppm barite had 11±0.7 μg sulfide/g soil while those that contained 10,000 ppm had 11±1.5 μg sulfide/g soil. The microbial activity was likely limited by the amount of electron donor (lactate) rather than the amount of electron acceptor (sulfate from barite).

Considering the soluble Ba^{++} release data obtained after 4 months incubation, the release in the viable culture supplemented with lactate and 2000 ppm barite was significantly greater than that in the sterile control ($P<0.05$) (Fig. 4.20). These values were 6.0±0.8 and 3.4±0.3 mg/kg soil, respectively. Similarly after 4 months incubation, the soluble Ba^{++} release in the viable culture supplemented with lactate and 10,000 ppm barite was significantly greater than that in the sterile control ($P<0.01$) (Fig. 4.21). These concentrations were 6.9±0.6 and 2.4±0.3 mg/kg soil, respectively (Fig. 4.21). Thus, the different amounts of barite added to the microcosms did not affect the final soluble Ba^{++} release in these microcosms. After 4 months incubation, those that contained 2000 ppm barite released 6.0±0.8 mg Ba^{++}/g soil while those that contained 10,000 ppm released 6.9±0.6 mg Ba^{++}/g soil. As was noted with the discussion of sulfide production, the microbial activity in these microcosms was likely limited by the amount of electron donor (lactate) rather than the amount of electron acceptor (sulfate from barite).

4.2.6 Methanogenic microcosms

At the time this study was being planned, it was speculated that the background soil would contain few, if any, methanogens. Thus, two sets of methanogenic microcosms were established. One set with background soil that was supplemented with 10 mL of domestic anaerobic sewage sludge, and one set with only the background soil. Both sets were supplemented with 2000 ppm barite.

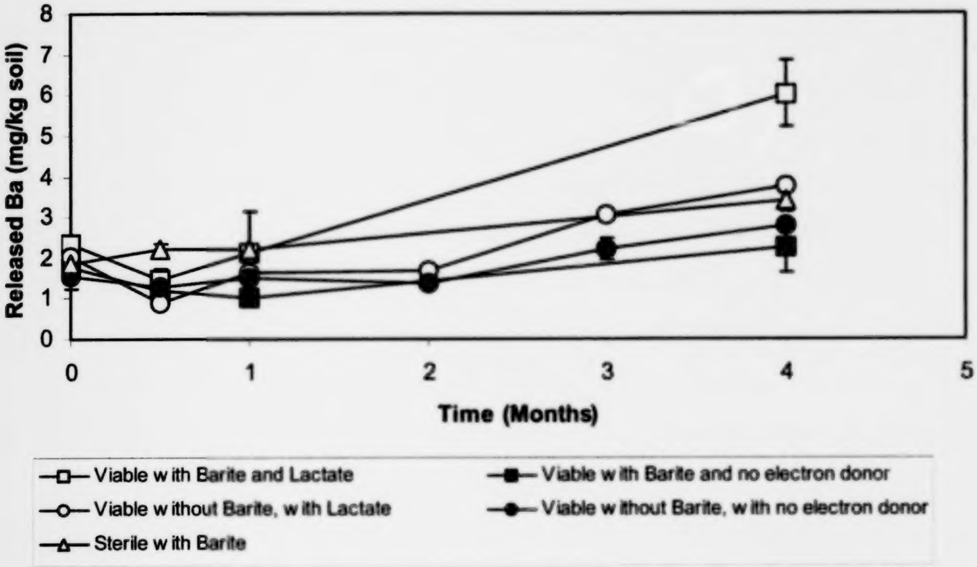

Fig. 4.20. Release of soluble Ba^{++} in the sulfate-reducing microcosms containing background soil supplemented with sulfate-reducing bacteria and 2000 ppm barite. Error bars show one standard deviation.

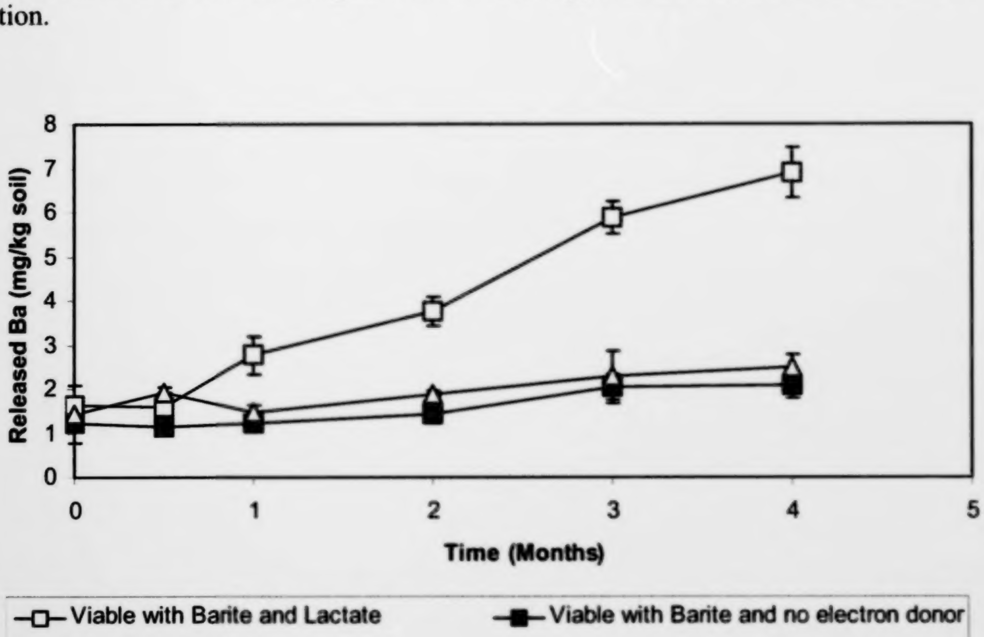

Fig. 4.21. Release of soluble Ba^{++} in the sulfate-reducing microcosms containing background soil supplemented with sulfate-reducing bacteria and 10,000 ppm barite. Error bars show one standard deviation.

4.2.6.1 Microcosms supplemented with anaerobic sewage sludge

As was observed with the other microcosms, glucose was consumed quickly in the methanogenic microcosms (Fig. 4.22). No glucose was detected in sewage sludge-supplemented methanogenic microcosms after 14 days of incubation. The viable microcosms were re-supplemented with glucose after 55, 65, and 107 days (1.8, 2.2, and 3.6 months, respectively) of incubation.

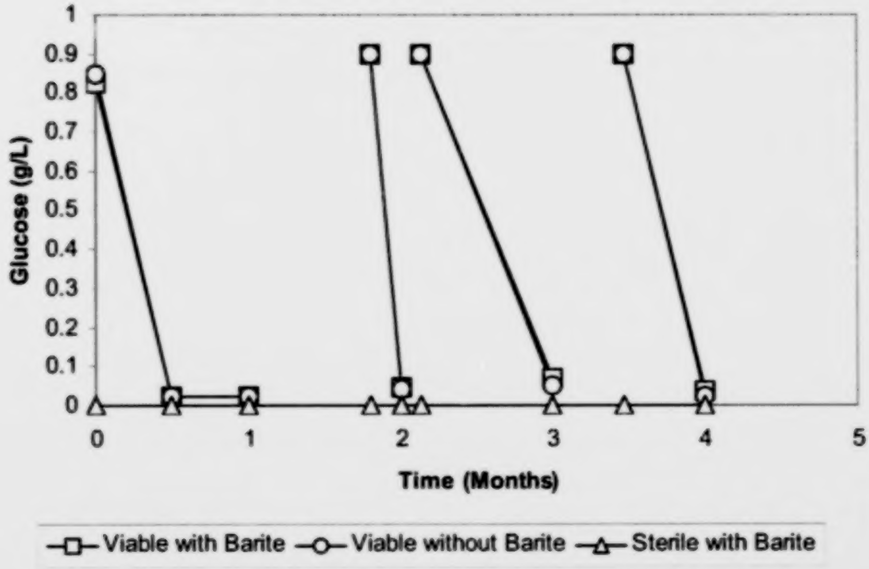

Fig. 4.22. Glucose concentrations in sewage sludge-supplemented microcosms. Glucose concentrations immediately after re-supplementing with glucose at 1.8, 2.2, and 3.6 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

Methane production was measured to follow the activity of the methanogens. The results are shown in Fig. 4.23 in which the amount of methane is given as its volume percent in the headspace gas. Methane was detected on day 1 in the microcosms that were supplemented with sewage sludge. The results in Fig. 4.23 clearly show that methane production was reduced in the microcosms that contained barite. It is well documented that sulfate-reducing bacteria out-compete methanogens for substrates (Banat et al. 1983; Winfrey and Ward 1983). Sulfate reduction is thermodynamically (Karhadkar et al. 1987) and kinetically (Kristjansson et al. 1982; Schönheit et al. 1982) more favorable than methanogenesis. The addition of the barite to the control soil provided an electron acceptor for the sulfate-reducing bacteria, and they have diverted the flow of electrons away from methane production. Thus, the methanogenic microcosms that were supplemented with barite were likely behaving as sulfate-reducing microcosms.

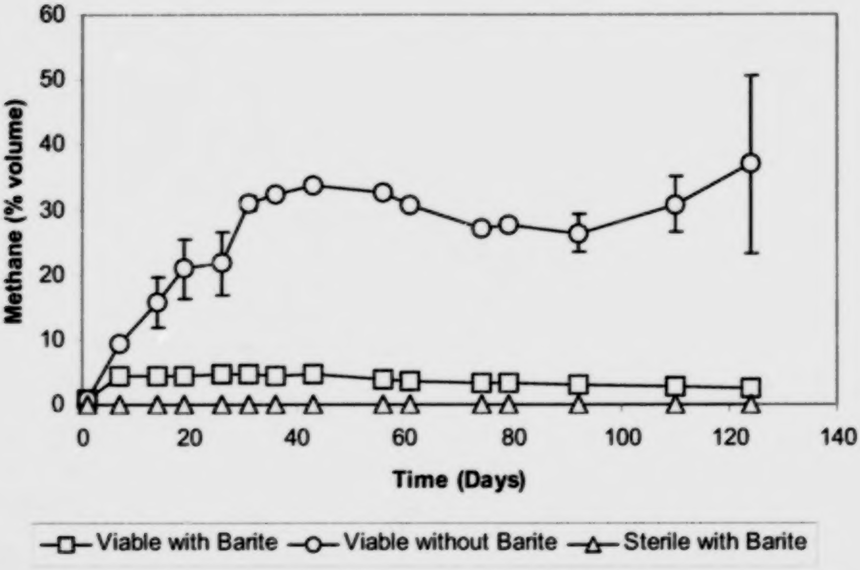

Fig. 4.23. Methane concentrations in sewage sludge-supplemented microcosms containing background soil. Error bars show one standard deviation.

The original plans for monitoring the methanogenic microcosms did not include measuring sulfide production. However, because of the observed inhibition of methane production caused by the addition of barite (Fig. 4.23), samples collected after 3 and 4 months of incubation were analyzed for sulfide. After 3 months of incubation, the average sulfide concentrations in the sterile, sewage sludge-supplemented methanogenic microcosms was 2.6±0.05 µg/g (wet weight), and in the viable sewage sludge-supplemented methanogenic microcosms, containing barite, was 6.0±0.7 µg/g (wet weight). There was a significant difference ($P<0.01$) between these means based on the t-test. The sulfide analyses clearly indicate that sulfate reduction occurred in the "methanogenic microcosms", and supports the notion that the sulfate-reducing bacteria were out-competing the methanogens for electron donors. However, after 4 months of incubation, the average sulfide concentrations in the sterile methanogenic microcosms was 3.8±0.06 µg/g (wet weight), and in the viable sewage sludge-supplemented methanogenic microcosms, containing barite, was 5.9±1.5 μg/g (wet weight). Because of the high variability in the results from the viable microcosms, the t-test indicated that the means were not significantly different.

Consistent with the reduction of sulfate in these microcosms, soluble Ba^{++} was released in the sewage sludge-supplemented microcosms that contained barite (Fig. 4.24). This was most obvious after 4 months of incubation when the average release was 5.4±0.3 mg/kg soil (wet weight) in the viable microcosms, and only 2.4±0 mg/kg soil (wet weight) in the sterile controls. Based on the t-test, there was a highly significant difference between these means ($P<0.001$). Thus, the results from the microcosms that were supplemented with anaerobic sewage sludge indicate that soluble Ba^{++} can be released from barite.

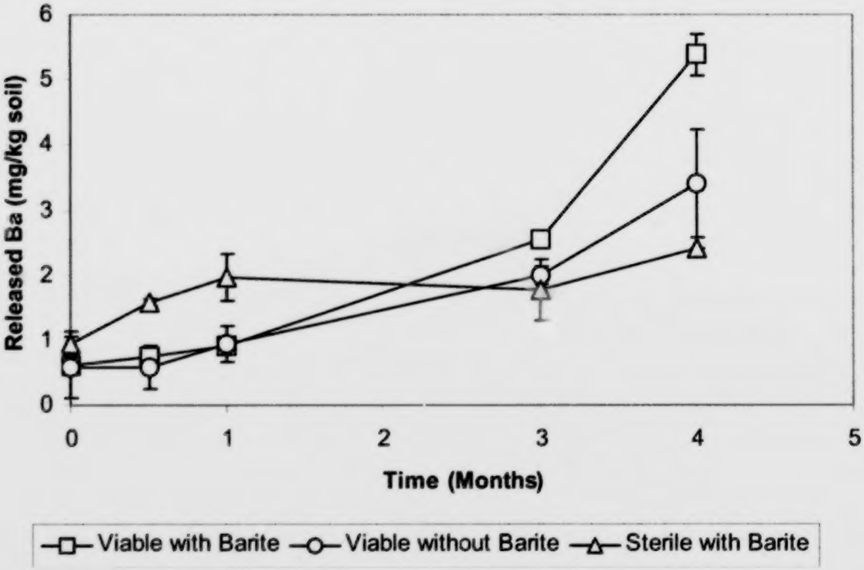

Fig. 4.24. Release of soluble Ba^{++} in the sewage sludge-supplemented microcosms containing background soil. Error bars show one standard deviation.

4.2.6.2 Microcosms with native methanogens only

Glucose was rapidly consumed in these methanogenic microcosms (Fig. 4.25) and by day 14, none was detected in the supernatant. The viable microcosms were re-supplemented with glucose after 55, 65, and 107 days (1.8, 2.2, and 3.6 months) of incubation.

The data in Fig. 4.26 showed that the background soil did contain methanogens, because methane production was evident after 40 days of incubation. The number of methanogens was likely quite low in this soil because of the long lag time before methane was observed compared to the microcosms that were supplemented with anaerobic sewage sludge in which methanogens were abundant. The lag period before methane was observed in the sewage sludge-supplemented microcosms was less than 1 day.

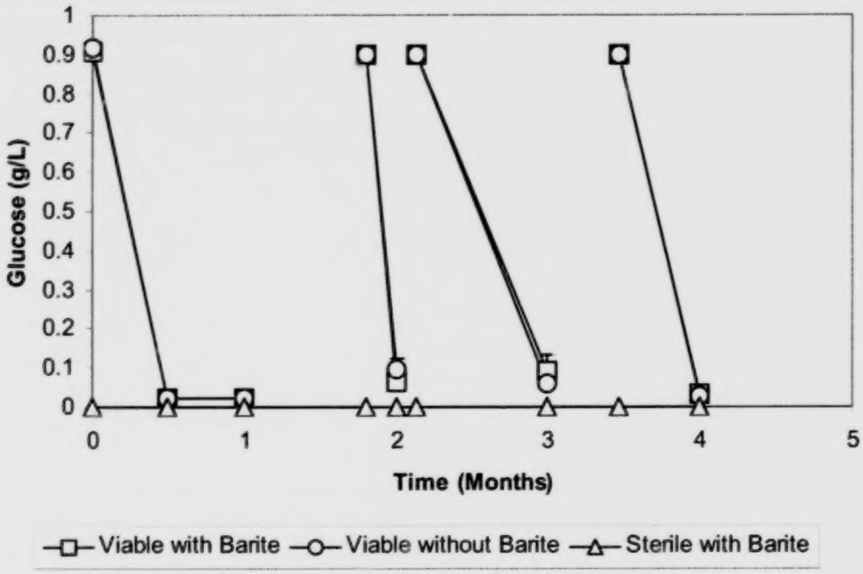

Fig. 4.25. Glucose concentrations in sewage sludge-supplemented microcosms. Glucose concentrations immediately after re-supplementing with glucose at 1.8, 2.2 and 3.6 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

There was a considerable amount of variability in the methane production summarized in Fig. 4.26. For example, on day 61, the methane measured in the duplicate microcosms without barite were 14% and 6% (by volume). This variability would be expected because it is difficult to obtain reproducible sub-samples from a soil with a low number of methanogens. However, by day 124 (the last sampling time in Fig. 4.26) there was virtually no difference in the methane concentrations in the duplicate microcosms without barite. After a long incubation period, the maximum methane yield is dependent on the amount of electron donor (glucose) that was supplied, rather than the initial numbers of methanogens in a sub-sample.

Very large error bars were observed with the average methane concentrations obtained from the viable microcosms supplemented with barite (Fig. 4.26). This is because methane production was completely inhibited in two of the triplicate microcosms. As was observed in Fig.

4.23, the addition of barite, a source of sulfate, severely inhibited methanogenesis. This is consistent with the sulfate-reducing bacteria out-competing the methanogens for electron donors. Because of the high rate of methane production in the third microcosm of the triplet set, it is possible that the barite was inadvertently omitted from this microcosm during the preparation and inoculation of the microcosm. However, this was not verified.

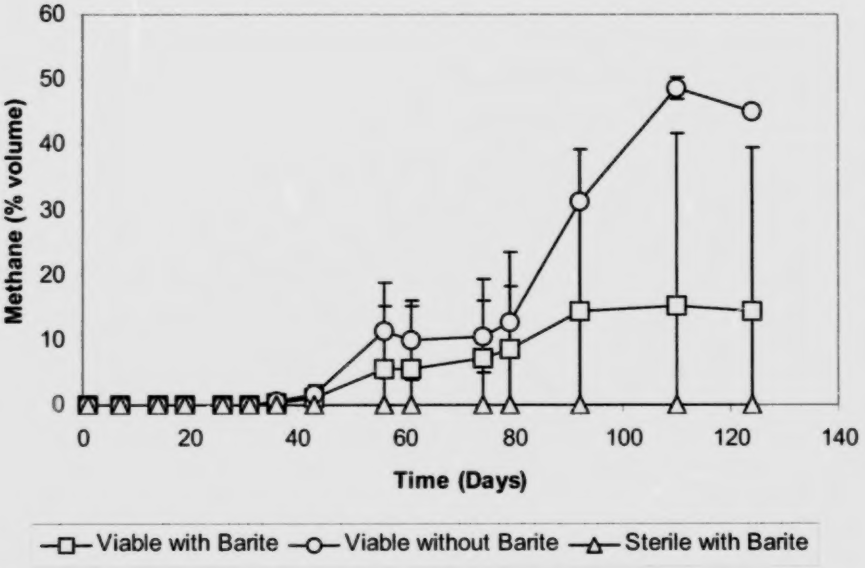

Fig. 4.26 Methane production in the microcosms containing background soil. Error bars are one standard deviation.

Because of the apparent activities of sulfate-reducing bacteria in the barite-supplemented microcosms, slurry samples collected at 3 and 4 months were analyzed for sulfide. After 3 months of incubation the average sulfide concentrations in the sterile, unsupplemented methanogenic microcosms was 2.5±0 µg/g (wet weight) and in the barite-containing viable microcosms was 4.2±0.7 µg/g (wet weight). Based on a t-test, there was no significant difference between these means ($p<0.01$). Similarly, there was no significant difference between the sulfide concentrations measured at 4 months. Thus, despite the strong inhibition of methanogenesis in two of the three microcosms supplemented with barite (Fig. 4.26), the enhanced activity of sulfate-reducing bacteria could not be demonstrated by measuring sulfide production, which was evident in the sulfate-reducing microcosms (Fig. 4.12).

The soluble Ba^{++} detected in the methanogenic microcosms that contained background soil is summarized in Fig. 4.27. Over the 4-month incubation period, there was a general increase in the amounts of soluble Ba^{++} in all of the microcosms. However, there was no statistical difference between the released Ba^{++} in the viable microcosms that received barite and the released Ba^{++} in the sterile controls after 4 months incubation. This is consistent with the results of the sulfide analyses. There was no increase in sulfide concentration in the barite-supplemented viable microcosms, so there should be no increase in released Ba^{++}. Thus, there was no evidence of Ba^{++} release in these methanogenic microcosms.

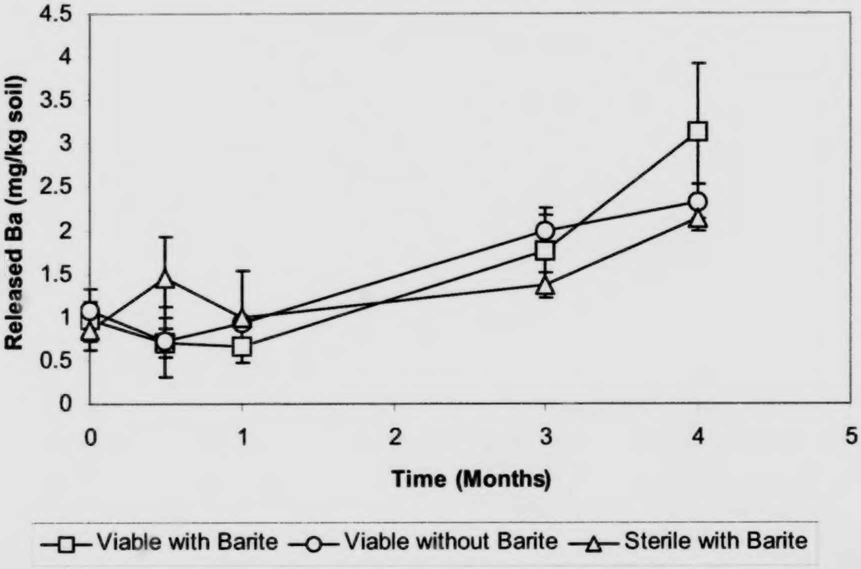

Fig. 4.27. Release of soluble Ba^{++} in the methanogenic microcosms containing background soil. Error bars show one standard deviation.

The results from these microcosms incubated under "methanogenic" suggest that soils which become heavily contaminated with barite may not become methanogenic because of the large supply of sulfate from the barite. This will stimulate the activities of sulfate-reducing bacteria that out-compete methanogens for electron donors.

5 SUMMARY AND CONCLUSIONS

The release of soluble Ba^{++} from barite was expected to be observed in the microcosms incubated under sulfate-reducing conditions. This was observed, indicating that the method used to establish the microcosms and method used for soluble Ba^{++} analysis were reliable. Table 5.1 is an abbreviated overview of the findings of this study. It includes only the results from those microcosms that were supplemented with glucose and 100 mg barite, and did not contain added microorganisms (such as the enrichment of sulfate-reducing bacteria or anaerobic sewage sludge).

Table 5.1 highlights that the release of soluble Ba^{++} from barite was observed in microcosms incubated under aerobic, Mn(IV)-reducing, Fe(III)-reducing and sulfate-reducing conditions. As discussed above, when barite was added to methanogenic microcosms, methanogenesis was inhibited, and sulfate reduction became the predominant microbial process. This would lead to the speculation that soluble Ba^{++} release from barite should have been observed in these microcosms, but it was not (Table 5.1). However, the release of soluble Ba^{++} from barite was observed in the methanogenic microcosms that were supplemented with anaerobic sewage sludge, and the addition of barite stopped methanogenesis (section 4.2.6.1). The reason for the discrepancy between these two sets of methanogenic microcosms is unclear.

The only microcosms in which there was a clear suppression of Ba^{++} dissolution from barite were those incubated under nitrate-reducing conditions. This was likely due to the well documented competition between nitrate-reducing bacteria and sulfate-reducing bacteria.

Table 5.1 also summarizes the maximum Ba^{++} release observed in the various microcosms, and includes ratios that express the extent of the increase in soluble Ba^{++}. Each ratio was calculated by dividing the maximum amount of soluble Ba^{++} in the microcosms by the initial amount of soluble Ba^{++} in the same microcosms. The greatest releases were found in the Fe(III)-reducing and sulfate-reducing microcosms, with values of 14 and 13 mg soluble Ba^{++}/kg soil (wet weight). The highest ratios of released Ba^{++} were in the Mn(IV)-reducing and Fe(III)-reducing microcosms. These showed 36-fold and 41-fold increases, respectively, in the amounts of soluble Ba^{++} under these incubation conditions. Part of the reason for these high ratios is because the initial soluble Ba^{++} concentrations were very low in these two sets of microcosms. The initial soluble Ba^{++} concentration was 31 μg/L in the Mn(IV)-reducing microcosms, and 160 μg/L in the Fe(III)-reducing microcosms. For comparison, the other initial soluble Ba^{++}

concentrations ranged from 230 μg/L in the methanogenic microcosms to 5,100 μg/L in the nitrate-reducing microcosms.

Table 5.1 Summary of the selected parameters from microcosms that contained background soil supplemented with glucose and 100 mg barite. The microbial activities were due to the native microorganisms only (results from microcosms with added microbes are not included).

Incubation condition	Ba^{++} release greater than in sterile control	Maximum Ba^{++} release (mg/kg soil)	Ratio: (Max. Ba^{++}/Time zero Ba^{++})
Aerobic	Yes[a]	8.1	1.7
Nitrate-reducing	No[b]	N/A[d]	N/A
Mn(IV)-reducing	Yes[c]	2.5	36
Fe(III)-reducing	Yes[a]	14	41
Sulfate-reducing	Yes[a]	13	7.6
Methanogenic	No[b]	N/A	N/A

[a] $P<0.01$

[b] No statistical difference between soluble Ba^{++} in the viable cultures and the sterile controls.

[c] $P<0.05$

[d] Not applicable because the amount of soluble Ba^{++} in the viable cultures was not statistically greater than the amount of soluble Ba^{++} in the sterile controls.

The findings of this short-term investigation, using microcosms with ample supplies of glucose as an electron donor suggest that there is a potential for barite dissolution releasing soluble Ba^{++} in soil environments that are dominated by aerobic, Mn(IV)-reducing, Fe(III)-reducing or sulfate-reducing conditions. The rates of microbial release of soluble Ba^{++} in these types of environments would likely be much slower than observed in this study, because the supply of electron donor would be rate limiting in the environment.

6 ACKNOWLEDGMENTS

We are indebted to Dr. G.J. Taylor for allowing us to use his AA spectrophotometer. We thank J. McDonald-Stephens for helping develop the AA procedure for barium analysis and we thank D. Coy for assisting with the preparation of the microcosms and many of the analytical methods. We also acknowledge technical assistance from Jessica Hrudey.

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8 APPENDIX

A.1 Aerobic medium (Sulfate-free – to prevent re-precipitation of $BaSO_4$) Fedorak and Coy (1996)

K_2HPO_4	0.5 g
NaCl	2.0 g
$MgCl_2$	0.2 g
KNO_3	2.0 g
NH_4Cl	1.0 g
$FeCl_2 \cdot H_2O$	trace
H_2O	1.0 L
Trace metals	1.0 mL

Trace metals (Fedorak and Grbic´-Galic´, 1991)

$CaCl_2 \cdot 2H_2O$	3.7 g
$ZnCl_2$	0.44 g
H_3BO_3	2.5 g
$MnCl_2$	0.87 g
$FeCl_3$	0.65 g
$Na_2MoO_2 \cdot 2H_2O$	0.29 g
$CoCl_2$	0.01 g
$CuCl_2$	0.0001 g
H_2O	1.0 L

A.2 General procedure for preparing anaerobic media

All media for anaerobic incubations were prepared using a 30% CO_2, 70% N_2 gas mixture. The media can be prepared in two ways:

1. Combine all of the ingredients except $NaHCO_3$ and vitamin solution and boil. Cool under gas mixture. When medium is cool, add $NaHCO_3$ and vitamin solution. Ensure that the pH is near neutral before dispensing into bottles.

2. Combine all of the ingredients. Bubble with gas mixture for at least 30 min before dispensing into bottles. Ensure that the pH is near neutral.

A.3 Preparation of sulfate-free, nitrate-reducing medium

K_2HPO_4	0.636 g
NaCl	2.0 g
NH_4Cl	0.06 g
Trace Metals (solution 2, section A.5)	1.0 mL
Wolfe's Vitamin Solution (section A.4)	1.0 mL
$NaHCO_3$	2.5 g
H_2O	1.0 L

The water was added immediately after it boiled. As the solution cooled, it was sparged with O_2-free gas. Then 100-mL portions were dispensed into ten 158-mL serum bottles that were flushed with O_2-free gas. These were sealed with rubber stoppers and autoclaved.

A solution of 20 mg/mL $CaCl_2$ and 20 mg/mL $MgCl_2$ was prepared by mixing the following in a serum bottle:

$CaCl_2 \cdot 2H_2O$	2.7 g
$MgCl_2 \cdot 6H_2O$	4.3 g
H_2O (Freshly boiled)	100 mL

This bottle was sealed with a rubber stopper and autoclaved.

After autoclaving, a 1-mL portion of the $MgCl_2$ and $CaCl_2$ solution was dispensed (using a syringe) into each bottle of nitrate-reducing medium.

A.4 Mn(IV) and Fe(III) reduction: (Lovley and Phillips, 1986, 1988)

$NaHCO_3$	2.5 g
$CaCl_2 \cdot H_2O$	0.1 g
KCl	0.1 g
NH_4Cl	0.06 g
$NaH_2PO_4 \cdot H_2O$	0.6 g
Wolfe's Vitamin Solution	1.0 mL
Wolfe's Mineral Solution	1.0 mL
H_2O	1.0 L

Slurry of MnO_2 or $Fe(OH)_3$*

*For Fe(III)-reducing medium, the $Fe(OH)_3$ slurry was prepared as outlined by Lovley and Phillips (1986). For the Mn(IV)-reducing medium, the MnO_2 slurry was prepared as outlined by Lovley and Phillips (1988). Fifteen millilitres of the appropriate slurry was added to 85 mL of medium.

Wolfe's Vitamin Solution
(ATCC, 1992)

Biotin	2.0 mg
Folic acid	2.0 mg
Pyroxidine-HCl	10.0 mg
Thiamine-HCl	5.0 mg
Riboflavin	5.0 mg
Nicotinic acid	5.0 mg
Na Pantothenate	5.0 mg
Cyanocobalamine	trace
p-Aminobenzoate	5.0 mg
Thioctic acid	5.0 mg
H_2O	100 mL

Wolfe's Mineral Solution
(ATCC, 1992)

Nitrilotriacetic acid	1.5 g
$MgSO_4 \cdot 7H_2O$	3.0 g
$MnSO_4 \cdot H_2O$	0.5 g
NaCl	1.0 g
$FeSO_4 \cdot 7H_2O$	0.1 g
$CoCl_2 \cdot 6H_2O$	0.1 g
$CaCl_2$	0.1 g
$ZnSO_4 \cdot 7H_2O$	0.1 g
$CuSO_4 \cdot 5H_2O$	0.01 g
$AlK(SO_4)_2 \cdot 12H_2O$	0.01 g
H_3BO_3	0.01 g
$Na_2MoO_4 \cdot 2H_2O$	0.01 g
$NiCl_2 \cdot 6H_2O$	0.025 g
H_2O	100 mL

A.5 Sulfate reduction: – Sulfate-free, $BaSO_4$ is the source of sulfate.

(Widdel-Pfennig medium - freshwater composition, Collins and Widdel, 1986)

Solution 1 (modified)	960 mL
Solution 2	1 mL
Solution 3	1 mL
Solution 4	30 mL
Solution 5	1 mL
Solution 6	1 mL

Solution 1 (Modified to be sulfate-free)

NaCl	1.0 g
$MgCl_2 \cdot 6H_2O$	0.4 g
NH_4Cl	0.1 g
KH_2PO_4	0.2 g
KCl	0.3 g
$CaCl_2 \cdot 2H_2O$	0.15 g
Resazurin (0.01 g/100 mL H_2O)	10 mL
H_2O	960 mL

Autoclave

Solution 2

HCl	4.5 mL
$FeCl_2 \cdot 4H_2O$	1.5 g
H_3BO_3	60 mg
$MnCl_2 \cdot 4H_2O$	100 mg
$CoCl_2 \cdot 6H_2O$	120 mg
$ZnCl_2$	70 mg
$NiCl_2 \cdot 6H_2O$	25 mg
$CuCl_2 \cdot 2H_2O$	15 mg
$Na_2MoO_4 \cdot 2H_2O$	25 mg
H_2O	995 mL

Autoclave

Solution 3

$Na_2SeO_3 \cdot 5H_2O$	3 mg
NaOH	0.5 g
H_2O	1.0 L

Autoclave

Solution 4

$NaHCO_3$	8.4 g
H_2O	100 mL

Saturate with CO_2, then filter sterilize

Solution 5

$Na_2S \cdot 9H_2O$	1.2 g
H_2O	10 mL

Boil H_2O

Solution 6

Biotin	10 mg
4-Aminobenzoic acid	40 mg
Thiamine-HCl	100 mg
B12	50 mg
Nicotinic acid	100 mg
H_2O	1.0 L

Filter sterilize

A.6 Methanogenic Medium (Fedorak and Hrudey, 1984)

Mineral Solution I	1.0 mL
Mineral Solution II	0.1 mL
Resazurin Solution	1.0 mL
Phosphate Solution	1.0 mL
$NaHCO_3$	0.57 g
H_2O	100 mL

Mineral Solution I

NaCl	5.0 g
$CaCl_2 \cdot H_2O$	1.0 g
NH_4Cl	1.0 g
$MgCl_2 \cdot 6H_2O$	1.0 g
0.01 M HCl	100 mL

Mineral Solution II

$(NH_4)_6Mo_7O_{24} \cdot 2H_2O$	1.0 g
$ZnSO_4 \cdot 7H_2O$	0.01 g
H_3BO_3	0.03 g
$FeCl_2 \cdot 4H_2O$	0.15 g
$CoCl_2 \cdot 6H_2O$	1.0 g
$MnCl_2 \cdot 4H_2O$	0.003 g
$NiCl_2 \cdot 6H_2O$	0.003 g
$AlK(SO_4)_2 \cdot 12H_2O$	0.01 g
H_2O	100 mL

Phosphate Solution

5.0 g KH_2PO_4 per 100 mL H_2O

Resazurin Solution

0.01 g per 100 mL H_2O

Appendix V

Barium Mobilization by Salt

APPENDIX V – BARIUM MOBILIZATION BY SALT

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1. INTRODUCTION

As part of the project to develop soil quality guidelines for barite, consideration was given to various conditions that could result in the mobilization of barium from barite. One of the conditions considered was the presence of high concentrations of salts, since releases of oilfield brine may have occurred in areas where oilfield barite is present. Typical oilfield brines are predominantly sodium chloride.

2. STUDY OBJECTIVE

The objective of this study was to determine whether the addition of sodium chloride to soil could release barium either from the soil or from barite added to the soil. It was intended that the range of salt concentrations used should be consistent with the range of electrical conductivities (ECs) typically found in soil at former oilfield facilities where brine releases have occurred (*i.e.*, up to 20 dS/m).

3. MATERIALS AND METHODS

3.1 Range Finding Experiment

Sixteen microcosms were set up as a range finding experiment to determine the relation between the amount of NaCl added and the EC of the supernatant. These sixteen microcosms contained approximately 50 g of soil (eight with contaminated soil and eight with background soil) and 100 mL of double distilled H_2O. 0 mg, 200 mg, 400 mg, 600 mg, 800 mg, 1,000 mg, 1,200 mg, and 1,400 mg NaCl were added to the eight flasks of background soil and this process was repeated for the contaminated soil. The flasks were covered in parafilm (to avoid evaporation) and placed on a shaker to shake for approximately 24 hours. After 24 hours, a small sample of each microcosm was placed in an Eppendorf Centrifuge tube and the sixteen tubes were centrifuged. Then the EC of the supernatant from these samples was measured. These results were used to generate standard curves (Figure II-1) for contaminated soil and for background soil to determine the mass of NaCl necessary to produce electrical conductivities of 0 dS/m, 2 dS/m, 4 dS/m, 8 dS/m and 20 dS/m.

3.2 Quantitative Experiment

Once the necessary amount of salt was determined to achieve the above-noted EC values, 20 microcosms were prepared: five microcosms contained approximately 50 g of background soil, 100 mL of double distilled H_2O and the respective amount of NaCl to produce the above-noted ECs; five microcosms were identical to the previous five, but each also contained approximately 100 mg of barite; five microcosms were identical to the first five, but each also contained 500 mg of barite; and five microcosms contained approximately 50 g contaminated soil, 100 mL of double distilled H_2O and the respective amounts of NaCl needed to generate the desired ECs.

These twenty microcosms were sealed with parafilm and placed on a shaker to shake for approximately 24 hours. After twenty-four hours 13 mL was removed from each sample and placed in large centrifuge tube and centrifuged for ten minutes. 1.4 mL of supernatant was transferred to Eppendorf tubes and stored for barium analysis. The remaining supernatant was used to measure the EC of each of the 20 microcosms.

4. RESULTS

The results of the quantitative experiment are summarized in Table V-1 and Figures V-1 and V-2.

5. CONCLUSIONS

The conclusions of this experiment are evident from Figure V-2 and are that adding salt to barite-contaminated soil can result in an increase in the soluble barium present, but that the barium released comes from the soil, and not from the barite. Accordingly, this process will not be significant when determining how much barite can safely remain in soil.

Table V-1. Barium Mobilization Data

	Mass of NaCl Added (mg)	Mass of Soil (g)	Mass of Barite Added (mg)	Measured EC (dS/m)	Soluble Ba^{2+} (mg/L)
Background Soil	0	50.15	0	0.22	0.02
	90.1	50.44	0	1.76	0.19
	232.9	50.13	0	3.7	0.41
	518.5	50.19	0	8.3	1
	1,375.70	50.17	0	19.6	3.4
Background + 2,000 mg/kg Barite	0	50.43	100.8	0.24	0.12
	90.1	50.52	99.9	1.74	0.23
	232.7	50.41	101.4	3.9	0.45
	518.7	50.21	101	8.3	0.96
	1,375.70	50.42	99.9	19.8	3.3
Background + 10,000 mg/kg Barite	0	50.54	499.8	0.26	0.11
	90.1	50.21	500.4	1.75	0.24
	232.9	50.56	500.7	3.9	0.65
	518.6	50.4	500	8.3	1
	1,375.60	50.02	500.4	19.7	2.9
Contaminated Soil	0	50.38	0	0.6	0.16
	64	50.6	0	1.64	0.11
	208.2	50.54	0	4.1	0.2
	496.1	50.2	0	8.4	0.37
	1,354.40	50.61	0	19.2	1

Figure V-1. Range Finding Test

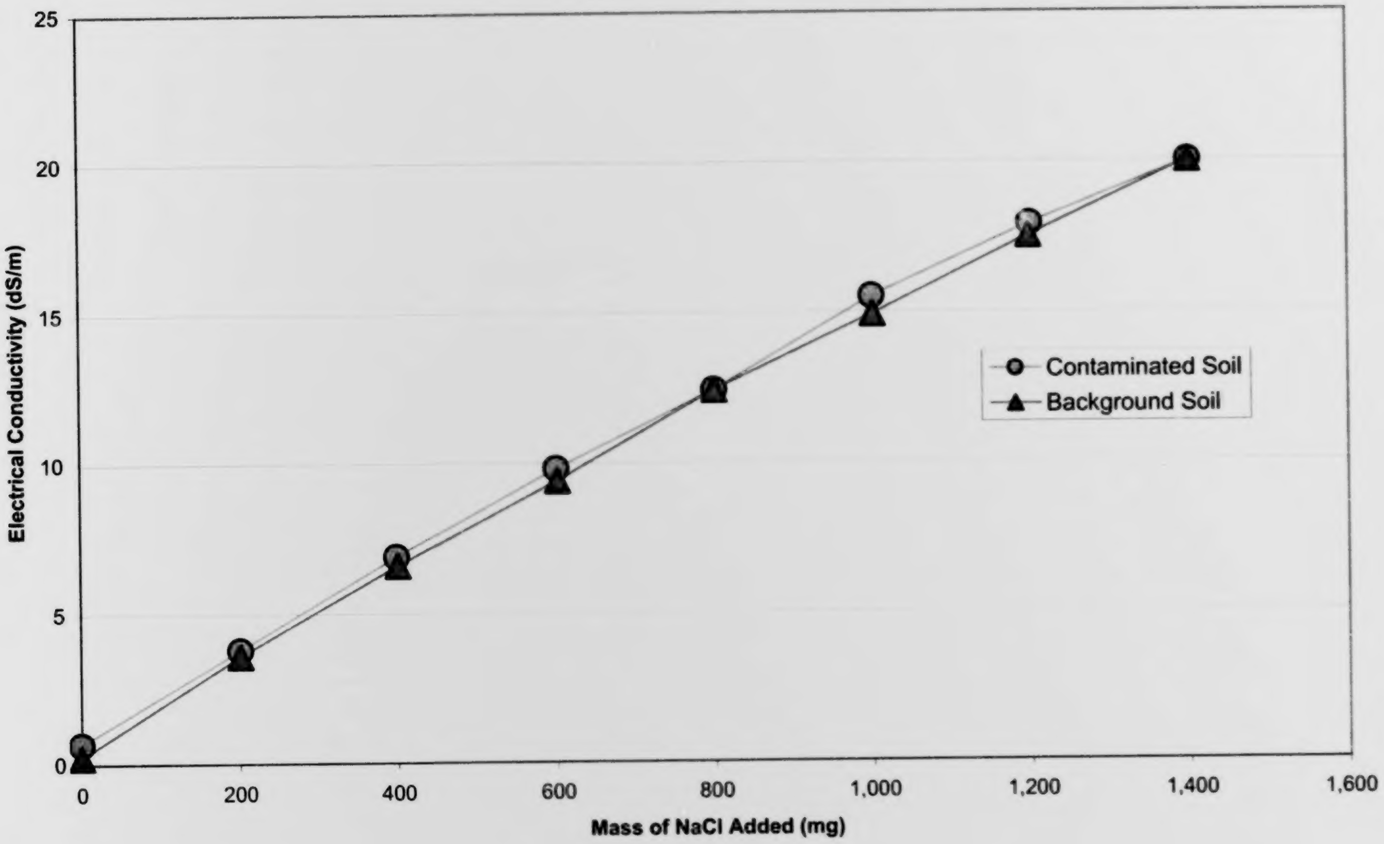
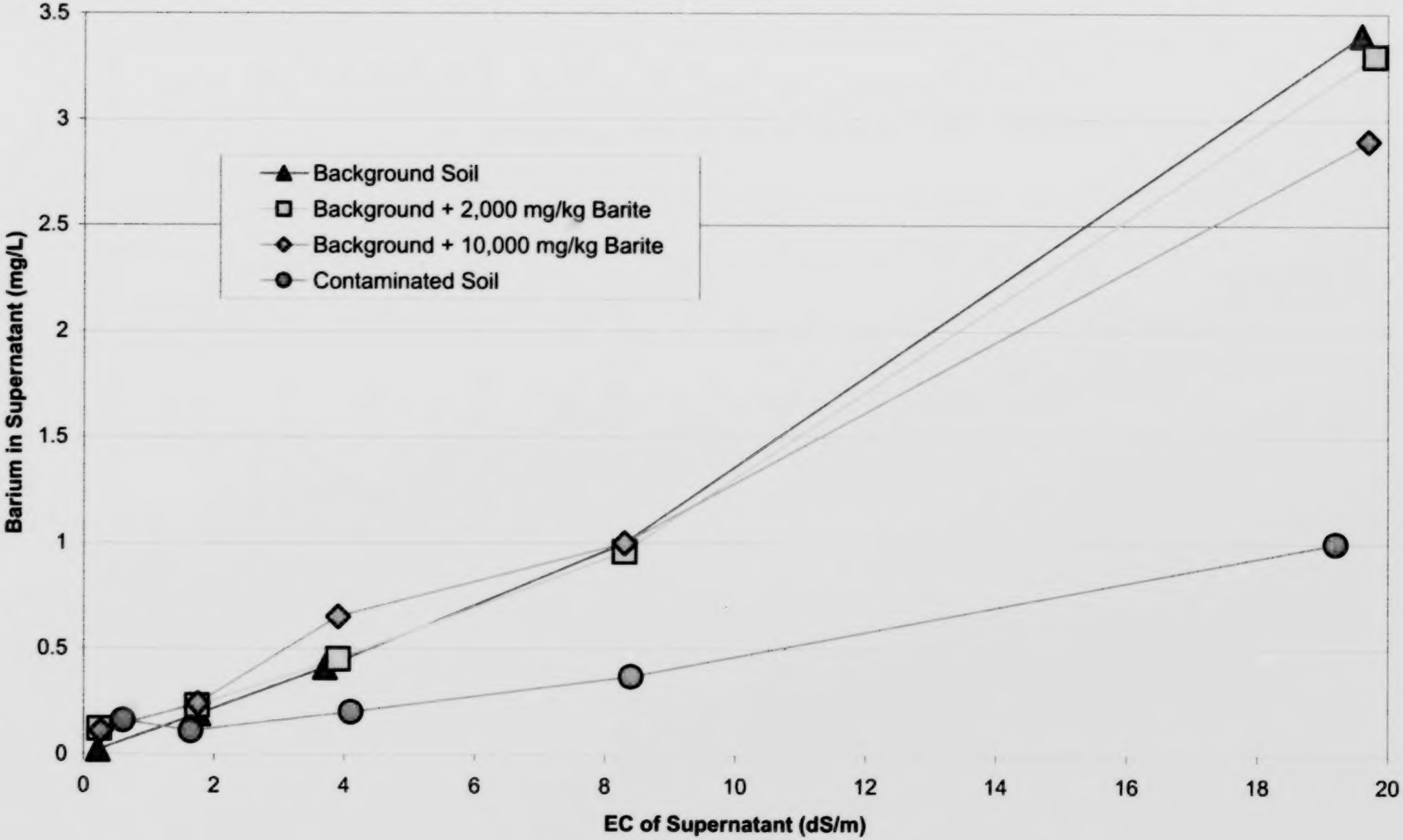


Figure V-2. Mobilization of Barium by Addition of NaCl



Appendix VI

ESG (2003a) –
Barite Ecotoxicity

ECOTOXICITY EVALUATION OF REFERENCE SITE SOILS AMENDED WITH BARIUM SULPHATE

Prepared for:

Miles Tindal
Komex International, Ltd.
4500-16th Avenue NW
Calgary, AB
T3B 0J1

Prepared by:

ESG International Inc.
361 Southgate Drive
Guelph, Ontario
N1G 3M5

G2144
January 2, 2003

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APPENDICES

Appendix A Results of the Amended Site Soil Assessment: Ecotoxicity Evaluation with Plant and Invertebrate Species

1. INTRODUCTION

In February 2002, ESG International was contracted by Komex International, Ltd. to provide an ecotoxicity assessment of site soils contaminated with a mixture of petroleum hydrocarbons and barium sulphate. The soils originated from an abandoned wellsite location near Pincher Creek, Alberta, and the site had been contaminated as a consequence of drilling activities (i.e., drilling mud disposal). The results of the acute earthworm test indicated that the contaminated site soil was not acutely toxic to earthworms; however, there was a 28 to 72% reduction in growth of two of the three plant species tested, following longer-term exposure to the contaminated soil, relative to the uncontaminated reference control soil. The barium sulphate soil concentration, as total barium sulphate, was at levels that exceeded federal (CCME) and Alberta provincial (Tier 1 - 1994) guideline criteria; however, the level of barium sulphate as saturated paste, which represents the water-soluble fraction, was extremely low. Petroleum hydrocarbon soil concentrations in the contaminated soil were at levels that exceeded the regulatory guideline criteria (CCME Fractions 2 and 3) and might have caused the adverse effects. As a result, Komex International contracted ESG to provide an ecotoxicity assessment of the previously collected reference control site soil amended with barium sulphate. The uncontaminated reference site soil is a very clayey soil collected by Komex International from a site in Alberta. The physical and chemical characteristics of the reference control soil were similar to those of the contaminated site soil, but free of contaminants.

A preliminary toxicity assessment (Phase 1) with barium sulphate-amended reference soil was conducted. It consisted of an acute earthworm toxicity test and an acute plant screening test. The test species were the earthworm, *Eisenia andrei*, and the plant alsike clover (*Trifolium hybridum*). The results of these preliminary tests were reported earlier (ESG 2002); they are also included in this report. A more comprehensive testing program (Phase 2) with higher barium sulphate soil concentrations was subsequently conducted and consisted of one acute earthworm test, one acute arthropod (springtail) test and three definitive plant tests. All experiments were conducted with undiluted background soil (e.g. a field-collected uncontaminated reference soil) amended with different quantities of industrial-grade barium sulphate, as well as with an experimental control soil (e.g. artificial soil). The earthworm and plant test species were identical to those used in the previous testing program with the contaminated site soil. The test species included the earthworm, *Eisenia andrei* (compost worm), the leguminous plant, alsike clover (*Trifolium hybridum*), and the monocotyledonous perennial ryegrass (*Lolium perenne*), and orchardgrass (*Dactylis glomerata*). These species had been originally chosen because *E. andrei* is commonly used to assess the effects of contaminated soil on terrestrial invertebrates, and the three plant species are used as forage crops on rangeland in Alberta. The arthropod species, a springtail, *Onychiurus folsomi*, was also included because it inhabits a niche in the terrestrial ecosystem different from that of earthworms and plants and it represents a different exposure pathway.

The soils used were those previously collected by Komex International and received by ESG on February 27, 2002. After their arrival at ESG's Southgate Laboratory, the soil samples (e.g., 20-L buckets) had been assigned identification numbers and were entered into a log book (Table A.1; Appendix A). Six buckets of the background soil had been received, and the samples were denoted as Background (1-6). Prior to testing, the soil samples had been air-dried for 24 hours, broken up by hand, pulverized into smaller aggregates, and sieved through a 6-mm stainless steel sieve. Once all the soil from the six buckets had been sieved, they were homogenized and were stored in plastic 20-L buckets at room temperature (20 ± 2°C) until use. The experimental control soil was an artificial soil (AS) formulated in the laboratory, and characterized as a fine sandy loam. The soil moisture contents of the background and artificial soils were determined prior to testing. The physical and chemical characteristics of the experimental control soil and the reference (background) control soil are listed in Table A.2 (Appendix A).

The objective of this research was to determine the concentration of barium sulphate in the reference control soil (background soil) that would cause an adverse effect on earthworm survival, springtail survival and seedling emergence and early growth. The experimental control soil provided QA/QC data on test organism health and experimental conditions. Preliminary testing began on July 30, 2002, and the more comprehensive testing program began on September 24, 2002.

2. PHASE 1: ACUTE TOXICITY TO *EISENIA ANDREI*

2.1 Experimental Design and Conditions

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- 14-day duration;
- Test endpoint: adult survivorship
- Temperature: constant at 20 ± 2°C; and,
- Photoperiod: 16-hr day, 8-hr night (fluorescent illumination).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background") soil, and batches of reference ("Background") soil amended with sufficient barium sulphate to achieve the following concentration series; 500, 1500, 5000, 10,000, 25,000, and 50,000 mg $BaSO_4$/kg soil dry weight (d.w.).

Test units consisted of 500-mL, wide-mouthed glass mason jars filled with 270 (AS) or 350 (background reference soil) g test soil wet weight (w.w.). Mass of soil per test unit varied depending on the bulk density of the soil. Jars were covered with perforated tin foil held by a metal screw ring to facilitate air exchange for the earthworms. Each treatment had 3 replicate test units, each with five adult earthworms. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998a).

2.2 Soil Preparation

On Day -1, the control and amended soils were prepared on a dry weight basis, but the volumes were adjusted to compensate for differences in the bulk density of the various soils. Barium sulphate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting $BaSO_4$-soil mixture was then hydrated with deionised water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the soils ranged from 13 to 37%. Physical and chemical soil parameters such as pH, electrical conductivity, and moisture content were measured following test soil preparation and at the end of the test (Table A.3; Appendix A).

On Day 0, five clitellate *Eisenia andrei* were added to each test unit and the soils in the test units were misted gently with deionised water as necessary. Substantially more water was added to the test units of the reference soil treatments after the earthworms were added to the test units, as compared to the experimental control soil treatment. The extremely clayey nature of the site soils precluded the addition of the required volume of deionised water while homogenizing the soil; therefore most of the water was added when the earthworms were added.

2.3 Statistical Analyses

No statistical analyses of the earthworm toxicity data were conducted because no mortality was observed. The data were tabulated instead.

2.4 Results

On Day 7, each test unit was inspected to determine the number and condition of surviving *E. andrei*. All earthworms in each treatment survived (n = 15), and all surviving worms appeared healthy (Table A.4; Appendix A).

On Day 14, each test unit was inspected again to determine the number and condition of surviving *E. andrei*. No mortality in any of the treatments occurred following 14 days of exposure, and all surviving worms appeared healthy (Table A.4; Appendix A). Soil pH, electrical conductivity and soil moisture content were measured and compared among the experimental control, reference control (0 mg $BaSO_4$/kg soil d.w.), and $BaSO_4$-amended reference soils. The addition of barium sulphate to the reference soil did not affect soil pH, and all soil pH values were within a range acceptable for *E. andrei* survival and growth. Soil moisture content (end of test) was comparable among all treatments, and was very similar among the reference soil treatments. Soil electrical conductivity in the reference soil was not affected by the addition of barium sulphate, and the electrical conductivity in all soil treatments was well within the range considered acceptable for *E. andrei* survival and growth (Table A.3; Appendix A).

3. PHASE 1: ACUTE SCREENING ALSIKE CLOVER TEST

3.1 Experimental Design and Conditions

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- Test organism was alsike clover from Pickseed Canada Inc., Sherwood Park, AB;
- Test duration: 14 d;
- Temperature: 24/16 ± 2°C day/night;
- Photoperiod: 16/8-h day/night ;
- Light intensity: ~ 400 • einsteins/m^2;
- Test endpoint: seedling emergence, shoot and root wet and dry mass.

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background") soil, and batches of reference ("Background") soil amended with sufficient barium sulphate to achieve the following concentration series; 500, 1500, 5000, 10,000, 25,000, and 50,000 mg $BaSO_4$/kg soil dry weight (d.w.).

Test units consisted of a glass petri dish enclosed within an inverted 1-L clear polypropylene container. Each petri dish was filled with either 70 g w.w. of AS at 35% moisture content or 100 g w.w. of reference ("Background") soil at 18% moisture content. More soil was added to test units containing the reference soil to compensate for differences in bulk densities between the soils. Both AS and reference soil treatments consisted of two replicate test units. Ten alsike clover seeds were planted in each test unit. Methods and procedures for site soil amendment are described in detail in EC, 1998b.

3.2 Soil Preparation

On Day 0, the control and amended soils were prepared on a dry weight basis, but volumes were adjusted to compensate for differences in bulk density of the soils. Barium sulphate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting $BaSO_4$-soil mixture was then hydrated with deionised water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the prepared test soils ranged from 13 to 35 %. In addition, the extremely clayey nature of the background soil precluded the addition of the optimal volume of deionised water while homogenizing the soil, therefore most of the water was added immediately after the seeds were planted. Physical and chemical soil parameters such as pH and electrical conductivity were measured following test soil preparation and at the end of the test (Table A.3; Appendix A). Seeds were planted in each test unit, to a depth of approximately the length of the longest side of the seed. Test units were then misted gently with deionised water. Substantially more water was added to the test units containing the clay background soil as compared to the experimental control soil. Test units were checked daily and watered as necessary with well-aerated de-chlorinated, municipal tap water.

3.3 Test Processing

On Day 14, each test unit was inspected to determine the number and condition of emerged seedlings. A seedling was considered emerged if the shoot tip was greater than three mm above the surface of the soil. Seedlings were carefully removed from the soil and any soil particles adhering to the roots were gently washed from each seedling using a spray bottle. Once cleaned, seedling shoot and root length were measured as well as shoot and root wet mass. Plant material was dried for 48 hours at 90°C after which shoot and root dry mass was also determined. The roots of alsike clover plants were inspected after length measurements were taken, and any obvious effect on the number of nodules present per plant, per treatment was recorded.

3.4 Statistical Analyses

Analysis of variance procedures were applied to the data followed by Dunnett's 2-sided pairwise comparison tests to compare treatment means of the amended reference soils to the control reference soil. To ensure that the assumptions of the analytical tests performed were met, the data and residuals were examined for normality and for homogeneity of variance among treatments. All analyses were performed with SYSTAT 7.0.1 (SPSS, 1997).

3.5 Results

Alsike clover emergence in the reference soil was not significantly affected by exposure to barium sulphate up to concentrations of 50,000 mg $BaSO_4$/kg soil d.w. (Figure A.1; Appendix A).

In general seedling growth also was not significantly affected by exposure to barium sulphate up to concentrations of 50,000 mg $BaSO_4$/kg soil d.w. Only one of the six endpoints (root length) was significantly lower than the root length of plants in the reference control soil (0 mg $BaSO_4$/kg soil d.w.) (Table A.5, Figure A.2; Appendix A), at the highest barium sulphate concentration tested (50,000 mg $BaSO_4$/kg soil d.w.). There were no obvious effects of exposure to barium sulphate on the abundance of nodules observed per plant per treatment.

Soil pH and electrical conductivity were measured and compared among the test soils, and the values for both parameters in all soil treatments were within the range that supports plant growth. The addition of barium sulphate to the reference soil did not affect pH or conductivity (Table A.3; Appendix A).

4. PHASE 2: CHEMICAL ANALYSES OF THE AMENDED SOIL

Prior to testing (Day 0), and at the end of testing (Day 14), the barium soil concentration of the BaSO4-amended soils was measured. Soil subsamples were collected from each spiked treatment (0 to 1,000,000 mg BaSO4/kg soil d.w.) at the beginning and end of the *Eisenia andrei* and perennial ryegrass Phase 2 tests. Samples were collected from one earthworm and one plant test to observe the effect, if any, of the presence of earthworms or plants on the total or water-soluble concentration of barium in the soil. Barium sulphate was analysed as total soil concentration and as saturated paste. Barium sulphate is sparingly soluble and, as a result, the $BaSO_4$-saturated paste values represent the water-soluble fraction of barium sulphate in the soil. The chemical analyses were performed by Philip Analytical Services Corporation, Mississauga, Ontario.

The total barium and barium as saturated paste concentrations in the soil samples collected from the *Eisenia andrei* and perennial ryegrass tests are presented in Table A.6 (Appendix A). There were no notable differences in any of the measured soil concentrations between the *E. andrei* and perennial ryegrass samples.

The reference control soil (0 mg/kg) had relatively high background total barium concentrations that ranged from 273 to 1160 ppm. Total barium soil concentrations in the lowest (1000 mg/kg) treatments ranged from 714 to 791 ppm. The measured concentrations are in general in agreement with the nominal concentration once background barium concentrations are accounted for, since 1000 g barium sulphate is equivalent to 588.4 g of barium. The total barium soil concentrations for all of the other nominal treatments however, were one to three orders of magnitude lower than expected. No concentration-response pattern was evident, and regardless of sampling time or source, the concentration of total measured barium was lowest at the highest nominal concentration (1,000,000 mg $BaSO_4$/kg soil d.w., or 100% $BaSO_4$). Despite this, the results were reasonably consistent; total soil concentrations were similar among the 10,000 to 1,000,000 mg/kg treatments, and ranged from 1060 to 1990 ppm. Within a nominal concentration, measured total barium concentrations were even more similar, particularly within the same sampling day. The analytical procedure used to measure total barium soil concentration was the Ontario Ministry of the Environment (OMOE) protocol E3073A, which is an aqua regia digest. OMOE protocol E3073A is the standard protocol used in Ontario to measure total soil metal concentrations. An aqua regia digest is an aggressive extraction procedure that uses hydrochloric and nitric acid to leach contaminants of interest from a sample. It is a standard extraction process used to extract inorganic contaminants, particularly metals, from soils. However, barium as the barium sulphate salt is not soluble in acid, therefore this particular method of extracting total barium from the soil samples was likely inappropriate.

The concentration of $BaSO_4$-saturated paste at Day 0 was below the detection limit (0.05 mg/L) in all samples from both tests, except for the highest concentration where it was still very low (0.11 to 0.30 mg/L). Barium was also detected (0.07 mg/L) in the control sample from the *E. andrei* test. Barium (as saturated paste) levels were higher in all the Day 14 samples and appeared to follow a consistent concentration–response pattern. The concentrations were still low, however, and ranged from 0.056 to 2.40 mg/L (Table A.6). This is consistent with the sparingly soluble nature of barium sulphate and suggests that it might be only slightly bioavailable to exposed soil organisms.

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5. PHASE 2: ACUTE TOXICITY TO *EISENIA ANDREI*

5.1 Experimental Design and Conditions

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- 14-day duration;
- Test endpoint: adult survivorship
- Temperature: constant at 20 ± 2°C; and,
- Photoperiod: 16-hr day, 8-hr night (fluorescent illumination).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background") soil, and batches of reference ("Background") soil amended with sufficient barium sulphate to achieve the following concentration series; 1000, 10,000, 30,000, 100,000, 300,000, and 1,000,000 mg $BaSO_4$/kg soil d.w.

Test units consisted of 500-mL, wide-mouthed glass mason jars filled with 270 (AS) to 350 (background reference soil) g soil wet weight (w.w.) of test soil. Mass of soil added per test unit varied depending on the bulk density of the soil. Jars were covered with perforated tin foil held by a metal screw ring to facilitate gas exchange for the earthworms. Each treatment included 3 replicate test units. Five adult earthworms were added to each test unit. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998a).

5.2 Soil Preparation

The test soils were prepared as described in Subsection 2.2.

5.3 Statistical Analyses

No statistical analyses of the earthworm toxicity data were conducted because there was no observed mortality; the results were simply tabulated.

5.4 Results

On Day 7, each test unit was inspected to determine the number and condition of surviving *E. andrei*. All earthworms in each treatment survived (n = 15), and all surviving worms appeared healthy (Table A.4; Appendix A).

On Day 14, each test unit was inspected again to determine the number and condition of surviving *E. andrei*. No mortality in any of the treatments occurred following 14 days of exposure, and all surviving worms appeared healthy (Table A.4; Appendix A). Soil pH, electrical conductivity and soil moisture content were measured and compared among the experimental control, reference control (0 mg $BaSO_4$/kg soil d.w.), and $BaSO_4$-amended reference soils. The addition of barium sulphate to the reference soil increased soil pH however, all soil pH values were within a range acceptable for *E. andrei* survival and growth. Soil moisture content (end of test) decreased with increasing concentrations of barium sulphate, however the soil moisture in all treatments was adequate for *E. andrei* survival and growth. Soil electrical conductivity in the reference soil was not affected by the addition of barium sulphate, and the electrical conductivity in all soil treatments was well within the range considered acceptable for *E. andrei* survival and growth (Table A.3; Appendix A).

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6. PHASE 2: ACUTE TOXICITY TO *ONYCHIURUS FOLSOMI*

6.1 Experimental Design and Conditions

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- 7-day duration;
- Test endpoint: adult survivorship
- Temperature: constant at 20 ± 2°C; and,
- Photoperiod: 16-hr day, 8-hr night (fluorescent illumination).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background") soil, and batches of reference ("Background") soil amended with sufficient barium sulphate to achieve the following concentration series; 1000, 10,000, 30,000, 100,000, 300,000, and 1,000,000 mg $BaSO_4$/kg soil d.w.

Test units consisted of 125-mL, wide-mouthed glass mason jars filled with 35 g soil wet weight (w.w.) of test soil. Jars were closed loosely with metal lids held by a metal screw ring to prevent escape of the organisms. Each treatment included 3 replicate test units. Ten mature springtails were added to each test unit. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998c).

6.2 Soil Preparation

On Day -1, the control and amended soils were prepared on a dry weight basis, but the volumes were adjusted to compensate for differences in the bulk density of the various soils. Barium sulphate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting $BaSO_4$-soil mixture was then hydrated with deionised water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the soils ranged from 8 to 33%. Physical and chemical soil parameters such as pH, electrical conductivity, and moisture content were measured following test soil preparation and at the end of the test (Table A.3; Appendix A).

On Day 0, ten mature *Onychiurus folsomi* were added to each test unit and the soils in the test units were misted gently with deionised water as necessary. More water was added to the test units of the reference soil treatments after the springtails were added to the test units, as compared to the experimental control soil treatment. The extremely clayey nature of the site soils precluded the addition of the required volume of deionised water while homogenizing the soil therefore most of the water was added when the springtails were added.

6.3 Statistical Analyses

No statistical analyses of the springtail toxicity data were conducted because there was no observed mortality; the data were simply tabulated.

6.4 Results

On Day 7, each test unit was inspected to determine the number and condition of surviving *O. folsomi*. All springtails in each treatment survived (n = 30), and all surviving springtails, as far as it was ascertainable, appeared healthy (Table A.7; Appendix A).

Soil pH, electrical conductivity and soil moisture content were measured and compared among the experimental control, reference control (0 mg $BaSO_4$/kg soil d.w.), and $BaSO_4$-amended reference soils. The addition of barium sulphate to the reference soil increased soil pH however all soil pH values were within a range acceptable for *O. folsomi* survival and growth. Soil moisture content (end of test) decreased with increasing concentrations of barium sulphate, however the soil moisture in all treatments was adequate for *O. folsomi* survival. Soil electrical conductivity in the reference soil was not affected by the addition of barium sulphate, and the electrical conductivity in all soil treatments was well within the range considered acceptable for *O. folsomi* survival (Table A.3; Appendix A).

7. PHASE 2: DEFINITIVE PLANT TOXICITY TESTS

7.1 Experimental Design and Conditions

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- Test organism was alsike clover, orchardgrass and perennial ryegrass from Pickseed Canada Inc., Sherwood Park, AB;
- Test duration: 14 d (orchardgrass and perennial ryegrass), 21 d (alsike clover);
- Temperature: 24/16 ± 2°C day/night;
- Photoperiod: 16/8-h day/night ;
- Light intensity: ~ 400 • einsteins/m^2;
- Test endpoint: seedling emergence, shoot and root wet and dry mass and, number of nodules per plant (alsike clover only).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background") soil, and batches of reference ("Background") soil amended with sufficient barium sulphate to achieve the following concentration series; 1000, 10,000, 30,000, 100,000, 300,000, and 1,000,000 mg $BaSO_4$/kg soil d.w.

Test units consisted of a 1-L clear polypropylene container. Each container was filled with either 485 g w.w. of AS at 35% moisture content or 375 g w.w. of reference ("Background") soil at 18% moisture content. More soil was added to test units containing the reference soil to compensate for differences in bulk densities between the soils. Both AS and reference soil treatments consisted of 4 replicate test units. Five orchardgrass and perennial ryegrass seeds, and ten alsike clover seeds were planted in each test unit. Methods and procedures for site soil amendment are described in detail in EC, 1998b.

7.2 Soil Preparation

Test soils were prepared as described in Subsection 3.2..

7.3 Test Processing

The test units were processed on Day 14 or 21 as described in Subsection 3.3. but, in addition, the roots of alsike clover plants were inspected after length measurements were taken, and the number of nodules present per plant was recorded.

7.4 Statistical Analyses

Analyses of variance procedures were applied to the data and a two-tailed Dunnett's test was used to compare each treatment mean to the mean of the reference (unamended) control soil treatment. The Dunnett's pairwise comparison test was used to compare treatment means of the amended reference soils to the control reference soil (SPSS, 1997). Where the data was amenable, linear or non-linear regression analyses were also conducted in order to generate IC50 and IC20 estimates. The analyses consisted of using a linear or four nonlinear regression models (i.e. logistic, gompertz, exponential and logistic with hormesis) that had been re-parameterized to include the ICp and the associated 95% confidence limits. The ICp is the inhibition concentration (IC) resulting in a specified percentage (p) effect. The data were examined for normality and homogeneity of variance among treatments. All analyses were performed with SYSTAT 7.0.1 (SPSS, 1997). A more detailed description of the statistical procedures used to analyze the definitive plant toxicity test data can be found in either Environment Canada (1998b) or Stephenson *et al*. (2000).

7.5 Results

7.5.1 Alsike clover

Alsike clover emergence in the reference soil was significantly decreased by exposure to barium sulphate at the highest concentration only (1,000,000 mg $BaSO_4$/kg soil d.w., or 100% $BaSO_4$) (Figure A.3; Appendix A). Pure (100%) barium sulphate is a very dense growth matrix for plants, and it is possible that the decreased emergence of alsike clover in this treatment was the result of a physical inhibition of emergence, rather than from chemical toxicity.

Barium sulphate in the reference soil in general did not adversely affect alsike clover seedling growth. Where there was a significant difference, seedling growth was greater in plants exposed to the barium sulphate-amended soils (Figures A.4 and A.5, Table A.9; Appendix A). Shoot length at the highest treatment concentration (1,000,000 mg $BaSO_4$/kg soil d.w.) was significantly lower relative to that in the control treatment. Of the six growth endpoints, only shoot dry mass data were amenable to regression analysis. Linear regression analysis of the shoot dry mass data estimated IC50 and IC20s greater than 1,000,000 mg/kg (100% barium sulphate) (Table A.8; Appendix A). Nodule production of alsike clover roots was not significantly affected by exposure to barium sulphate-amended soils (Figures A.6 and A.7; Appendix A).

7.5.2 Orchardgrass

Orchardgrass seedling emergence in the reference soil was not significantly affected by exposure to barium sulphate at concentrations up to 1,000,000 mg $BaSO_4$/kg soil d.w. (Figure A.3; Appendix A).

Barium sulphate in the reference soil did not adversely affect orchardgrass seedling growth. Where there was a significant difference in growth compared to the control treatment, seedling growth was greater in the barium sulphate-amended soils (Figures A.8 and A.9, Table A.9; Appendix A). Of the six growth endpoints measured, only root length data were amenable to regression analysis. Linear regression analysis of the root length data estimated IC50 and IC20s of > 1,000,000 and 850,000 mg/kg, respectively (Table A.8). Of all the endpoints, only root length appeared to be somewhat affected.

7.5.3 Perennial ryegrass

Perennial ryegrass seedling emergence in the reference soil was not significantly affected by exposure to barium sulphate at concentrations up to 1,000,000 mg $BaSO_4$/kg soil d.w. (Figure A.3; Appendix A).

Barium sulphate in the reference soil in general did not adversely affect perennial ryegrass seedling growth. Shoot growth was significantly greater in the reference soil amended with 1,000,000 mg $BaSO_4$/kg soil d.w. (Figures A.10 and A.11, Table A.9; Appendix A). Root length was significantly decreased following exposure to barium sulphate-amended soils only at the highest treatment concentration (1,000,000 mg $BaSO_4$/kg soil d.w.). Of the six growth endpoints measured, only root length data were amenable to regression analysis. Non-linear regression analyses of the root length data estimated IC50 and IC20s of >1,000,000 and 960,000 mg/kg, respectively (Table A.8; Appendix A).

7.5.4 Soil physical and chemical characteristics

As with the earthworm and collembola Phase 2 test soils, the addition of barium sulphate to the reference soil in all three plant tests increased soil pH, however all soil pH values were within the range that supports plant growth. Soil moisture content (beginning of test) decreased with increasing concentrations of barium sulphate; however water was added to increase moisture levels to close to saturation after the seeds were planted. Electrical conductivity of the reference soil was not affected by the addition of barium sulphate, and the electrical conductivity of all soil treatments was well within the range that supports plant growth (Table A.3; Appendix A).

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8. DISCUSSION

The results from both the Phase 1 and Phase 2 tests suggest that barium sulphate is not acutely toxic to the terrestrial invertebrates, even at nominal concentrations as high as 1,000,000 mg $BaSO_4$/kg soil d.w. Barium sulphate was not acutely toxic to alsike clover up to 50,000 mg $BaSO_4$/kg soil d.w.. The results from the Phase 2 definitive toxicity testing with alsike clover, orchardgrass, and perennial ryegrass indicate that longer-term exposure to barium sulphate did not result in toxicity to the three plant species, even at the highest exposure concentrations possible (100% $BaSO_4$ or 1,000,000 mg/kg soil d.w.). The amendment of barium sulphate to the reference control soil did not substantially alter the physical and chemical characteristics of the soil.

Natalie

Natalie Feisthauer, Project Manager

Gladys L Stephenson

Gladys Stephenson, Project Director

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APPENDIX A

RESULTS OF THE AMENDED SITE SOIL ASSESSMENT: ECOTOXICITY EVALUATION WITH PLANT AND INVERTEBRATE SPECIES

Table A.1. Identification and description of the Alberta reference site soils received from Komex International.

Sample	Date Received	Assigned Sample Identification	Observations
Background Reference Soil	27/02/2002	G2144 Background-1	Light yellow/grey clay, extremely chunky, and moist to dry.
Background Reference Soil	27/02/2002	G2144 Background-2	Same as G2144 Background-1
Background Reference Soil	27/02/2002	G2144 Background-3	Same as G2144 Background-1
Background Reference Soil	27/02/2002	G2144 Background-4	Same as G2144 Background-1
Background Reference Soil	27/02/2002	G2144 Background-5	Same as G2144 Background-1
Background Reference Soil	27/02/2002	G2144 Background-6	Same as G2144 Background-1

Table A.2. Physico-chemical characteristics of the experimental control soil (artificial soil, AS).

Parameter	Artificial Soil (AS)	Background Reference Soil (RS)	Analytical Method
Phosphorous (mg/kg)	23	< 1	Nitric/perchloric acid digestion
Potassium (mg/kg)	22	194	NH_4Ac extractable
Magnesium (mg/kg)	149	846	NH_4Ac extractable
Calcium (mg/kg)	1848	4026	NH_4Ac extractable
Sodium (mg/kg)	67	73	NH_4Ac extractable
Total Carbon (%)	4.46	1.23	Leco furnace method
Total Nitrogen (%)	0.05	0.07	Kjeldahl method
C.E.C. (Cmol+/kg)	18.5	23.0	Barium chloride method
Soil Texture	Fine Sandy Loam	Clay Loam	Gravimetric grain size distribution
Sand (%)	77.3	29.9	
Silt (%)	7.8	40.7	
Clay (%)	14.9	29.5	
Organic Matter (%)	9	1.2	Dichromate oxidation
Bulk Density (g/cm^3)	0.98	1.28	Clod method
pH (units)	6.09	7.7	Water method (1:2)
Conductivity (mS/cm)	0.3	0.54	Saturated paste method
Soil Moisture (%) at Field Capacity (0.3 bar)	N/A	24.4	Gravimetric analysis
Source	Formulated from constituents	Alberta	

C.E.C. Cation Exchange Capacity

Table A.3. Physico-chemical characteristics of treatment soils (artificial soil, reference control and amended site soils) from Phase 1 and 2 testing, following test preparation and processing.

Test	Treatment (mg $BaSO_4$/kg reference soil d.w.)	Initial pH[1]	Final pH[2]	Initial Conductivity (µS/m)	Final Conductivity (µS/cm)	Initial Moisture Content (%)	Final Moisture Content (%)
Phase 1 Testing							
Eisenia andrei	Artificial Soil	6.57	5.92	144	166	37.1	36.9
	Reference Soil	7.90	7.84	201	188	15.1*	24.5
	500	7.98	7.90	203	203	12.8*	23.1
	1500	8.02	7.96	214	207	12.9*	22.6
	5000	8.03	7.99	223	212	15.1*	22.6
	10,000	8.01	8.02	215	202	15.4*	23.5
	25,000	7.98	7.99	224	211	15.0*	23.3
	50,000	7.99	8.04	235	215	14.3*	22.2
Alsike Clover	Artificial Soil	6.57	7.45	144	214	[3]	[3]
	Reference Soil	7.90	8.26	201	231	[3]	[3]
	500	7.98	8.33	203	216	[3]	[3]
	1500	8.02	8.29	214	238	[3]	[3]
	5000	8.03	8.32	223	224	[3]	[3]
	10,000	8.01	8.33	215	246	[3]	[3]
	25,000	7.98	8.31	224	275	[3]	[3]
	50,000	7.99	8.36	235	233	[3]	[3]
Phase 2 Testing							
E. andrei	Artificial Soil	6.91	6.76	192	203	32.7	35.6
	Reference Soil	7.93	8.08	204	200	14.1*	20.6
	1000	8.03	8.19	206	204	15.3*	20.9
	10000	8.03	8.15	216	215	13.6*	20.3
	30000	8.01	8.23	220	223	13.7*	19.2
	100000	8.04	8.27	220	222	14.0*	19.3
	300000	8.08	8.28	228	226	13.2*	18.9
	1000000	8.39	8.48	220	187	8.0*	10.8
O. folsomi	Artificial Soil	6.91	6.48	192	219	32.7	32.6
	Reference Soil	7.93	7.85	204	208	14.1*	17.3
	1000	8.03	7.8	206	220	15.3*	18.7
	10000	8.03	7.87	216	221	13.6*	18.5
	30000	8.01	7.85	220	225	13.7*	17.7
	100000	8.04	8.03	220	219	14.0*	14.7
	300000	8.08	8.04	228	218	13.2*	16.2
	1000000	8.39	8.21	220	212	8.0*	9.8
Alsike Clover	Artificial Soil	7.54	7.97	221	436	31.9	[3]
	Reference Soil	7.94	8.02	193	480	15.8	[3]
	1000	7.98	8.12	214	402	13.1	[3]
	10000	7.99	8.17	228	464	15.9	[3]
	30000	8	8.25	219	428	15.6	[3]
	100000	7.99	8.18	222	478	14.8	[3]
	300000	8.01	8.32	230	490	13.4	[3]
	1000000	8.3	9.06	220	439	8.2	[3]

Table A.3. Physico-chemical characteristics of treatment soils (artificial soil, reference control and amended site soils) from Phase 1 and 2 testing, following test preparation and processing.

Test	Treatment (mg $BaSO_4$/kg reference soil d.w.)	Initial pH[1]	Final pH[2]	Initial Conductivity (µS/m)	Final Conductivity (µS/cm)	Initial Moisture Content (%)	Final Moisture Content (%)
Orchardgrass	Artificial Soil	7.52	7.71	220	385	34.0	[3]
	Reference Soil	7.66	8.04	195	407	15.3	[3]
	1000	7.86	8.09	215	428	15.9	[3]
	10000	7.92	8.12	231	373	14.5	[3]
	30000	7.94	8.09	232	472	15.7	[3]
	100000	7.97	8.11	227	490	14.1	[3]
	300000	8.01	8.11	232	621	13.8	[3]
	1000000	8.32	8.58	233	434	8.4	[3]
Perennial Ryegrass	Artificial Soil	7.54	8.16	221	301	31.9	[3]
	Reference Soil	7.94	8.29	193	343	15.8	[3]
	1000	7.98	8.29	214	364	13.1	[3]
	10000	7.99	8.26	228	436	15.9	[3]
	30000	8	8.26	219	449	15.6	[3]
	100000	7.99	8.27	222	455	14.8	[3]
	300000	8.01	8.41	230	293	13.4	[3]
	1000000	8.3	8.65	220	443	8.2	[3]

[1] measured at the beginning of the test

[2] measured at the end of the test

[3] moisture content measurements are not usually taken when conducting plant tests since irrigation changes the moisture content of the soil

*deionized water was added after soil m.c. measurements were taken at the beginning of the test

Table A.4. *Eisenia andrei* adult survival following 7 and 14 days of exposure to artificial soil, reference control site soil, and $BaSO_4$-amended reference site soil.

Treatment	Day 7		Day 14	
(mg $BaSO_4$/kg reference soil d.w.)	Survival (n = 15)	Standard Error	Survival (n = 15)	Standard Error
Phase 1 Testing				
Artificial Soil	15	0	15	0
Reference Soil	15	0	15	0
500	15	0	15	0
1500	15	0	15	0
5000	15	0	15	0
10,000	15	0	15	0
25,000	15	0	15	0
50,000	15	0	15	0
Phase 2 Testing				
Artificial Soil	15	0	15	0
Reference Soil	15	0	15	0
1000	15	0	15	0
10000	15	0	15	0
30000	15	0	15	0
100000	15	0	15	0
300000	15	0	15	0
1000000	15	0	15	0

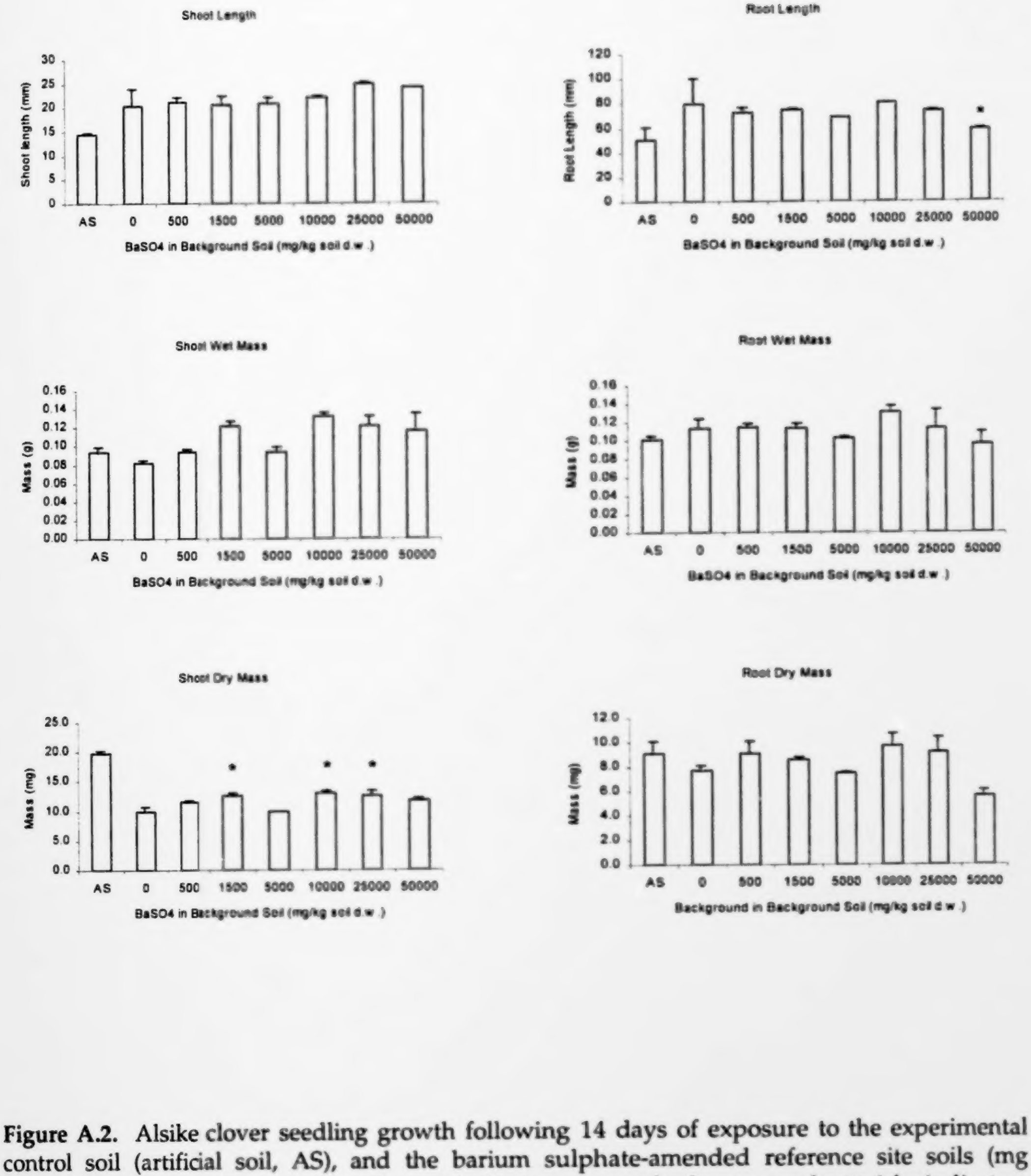


Figure A.2. Alsike clover seedling growth following 14 days of exposure to the experimental control soil (artificial soil, AS), and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars on the columns indicate one standard error, and asterisks indicate a significant difference from the reference control site soil.

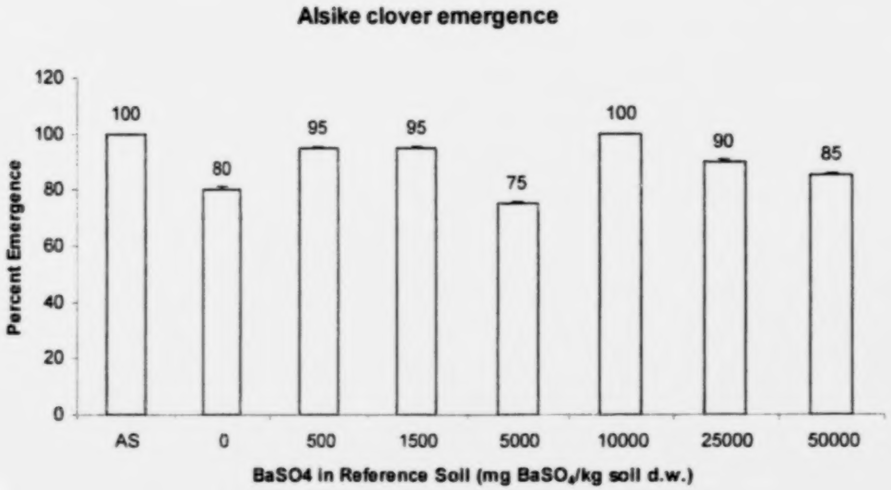


Figure A.1. Emergence of alsike clover following 14 days of exposure to the experimental control soil (artificial soil, AS), and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars indicate one standard error, and numbers above the columns indicate the percent emergence for each treatment.

Table A.5. The effect of exposure of alsike clover to barium sulphate-amended reference site soil, reference control soil, and artificial soil (Phase 1 test). Values in brackets indicate one standard error. Asterisks indicate values significantly different from those of the reference control soil treatment.

Treatment (mg BaSO4/kg reference soil d.w.)	**Endpoint**					
	Shoot Length (mm)	**Shoot Wet Mass** (g)	**Shoot Dry Mass** (mg)	**Root Length** (mm)	**Root Wet Mass** (g)	**Root Dry Mass** (mg)
Artificial Soil	14.60 (0.20)	0.09 (0.01)	19.90 (0.40)	50.70 (9.40)	0.10 (0.004)	9.10 (1.00)
Reference Soil	20.33 (3.44)	0.08 (0.03)	9.95 (0.75)	79.21 (21.06)	0.11 (0.01)	7.75 (0.35)
500	21.18 (0.96)	0.09 (0.003)	11.65 (0.15)	72.17 (4.21)	0.11 (0.004)	9.10 (1.00)
1500	20.70 (1.59)	0.12 (0.01)	12.50* (0.60)	73.91 (1.33)	0.11 (0.01)	8.65 (0.15)
5000	20.80 (1.38)	0.09 (0.01)	9.90 (0)	68.59 (0.17)	0.10 (0.0003)	7.45 (0.15)
10,000	22.15 (0.30)	0.13 (0.004)	13.05* (0.35)	79.95 (0.30)	0.13 (0.01)	9.75 (0.95)
25,000	24.84 (0.30)	0.12 (0.01)	12.70* (0.70)	73.24 (1.31)	0.11 (0.02)	9.20 (1.20)
50,000	24.27 (0.07)	0.12 (0.02)	11.85 (0.25)	58.05* (1.40)	0.10 (0.01)	5.60 (0.50)

Table A.6. Chemical analyses of the barium sulphate-amended reference soil.

Treatment (mg $BaSO_4$/kg soil d.w.)	**Total Barium*** (ppm)		**Barium as Saturated Paste** (mg/L)	
	Day 0	**Day 14**	**Day 0**	**Day 14**
Eisenia andrei				
Reference Soil	273	289	0.07	0.056
1000	720	714	<0.05	0.041
10000	1920	1570	<0.05	Not available
30000	1990	1630	<0.05	0.061
100000	2080	1620	<0.05	0.069
300000	1950	1330	<0.05	0.231
1000000	1470	1060	0.11	1.92
Perennial Ryegrass				
Reference Soil	284	1160	<0.05	0.172
1000	766	791	<0.05	0.073
10000	1830	1440	<0.05	0.071
30000	1910	1520	<0.05	0.055
100000	1830	1410	<0.05	0.067
300000	1570	1150	<0.05	0.134
1000000	1230	1060	0.30	2.40

*Samples analysed by ICAP, which is an acid digestion. Barium sulphate is not soluble in acid, therefore the analytical values underestimate real soil $BaSO_4$ concentrations.

Table A.7. *Onychiurus folsomi* survival following 7 days of exposure to artificial soil, reference control site soil, and $BaSO_4$-amended reference site soil.

Treatment (mg $BaSO_4$/kg reference soil d.w.)	Survival (n = 30)	Standard Error
Artificial Soil	30	0
Reference Soil	30	0
1000	30	0
10000	30	0
30000	30	0
100000	30	0
300000	20*	0
1000000	30	0

* N = 20 for this treatment (one replicate test unit was destroyed)

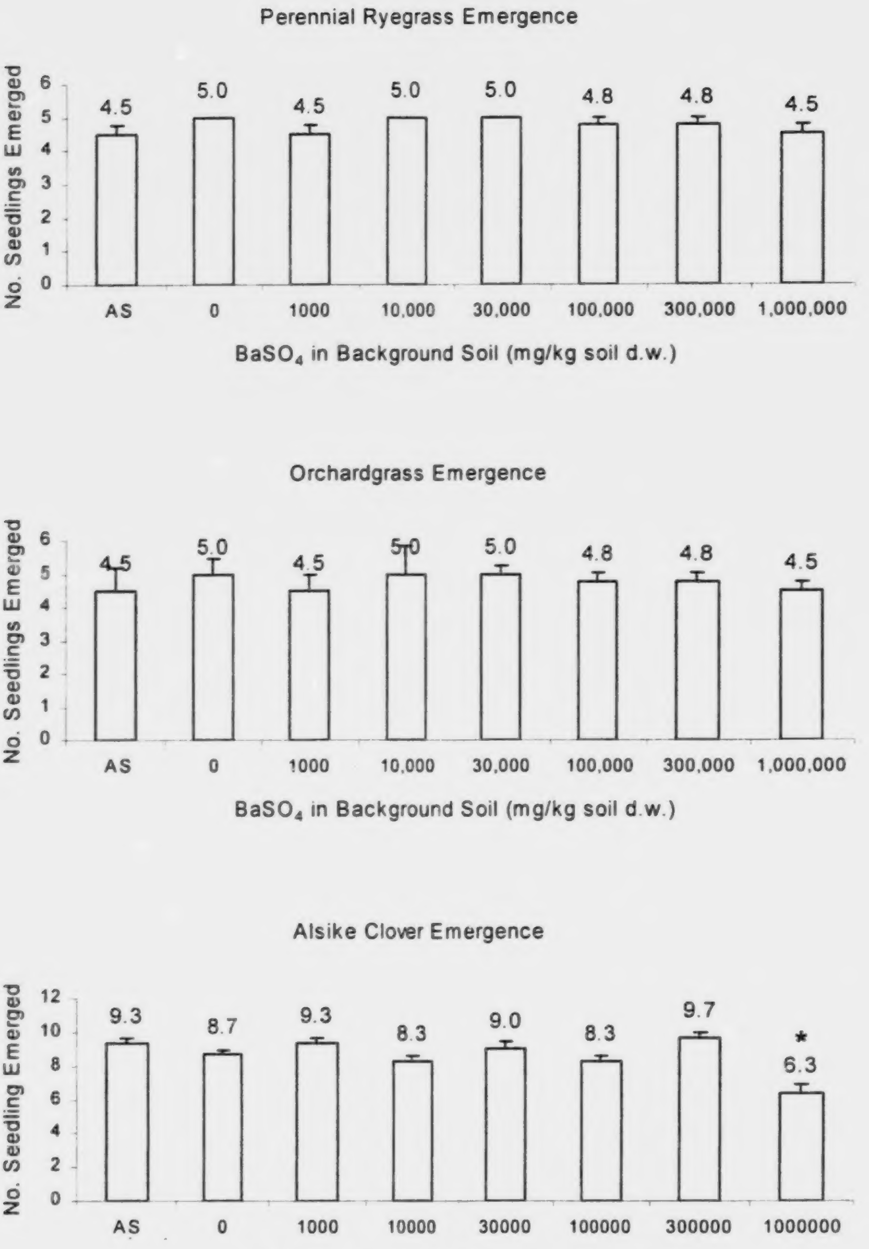
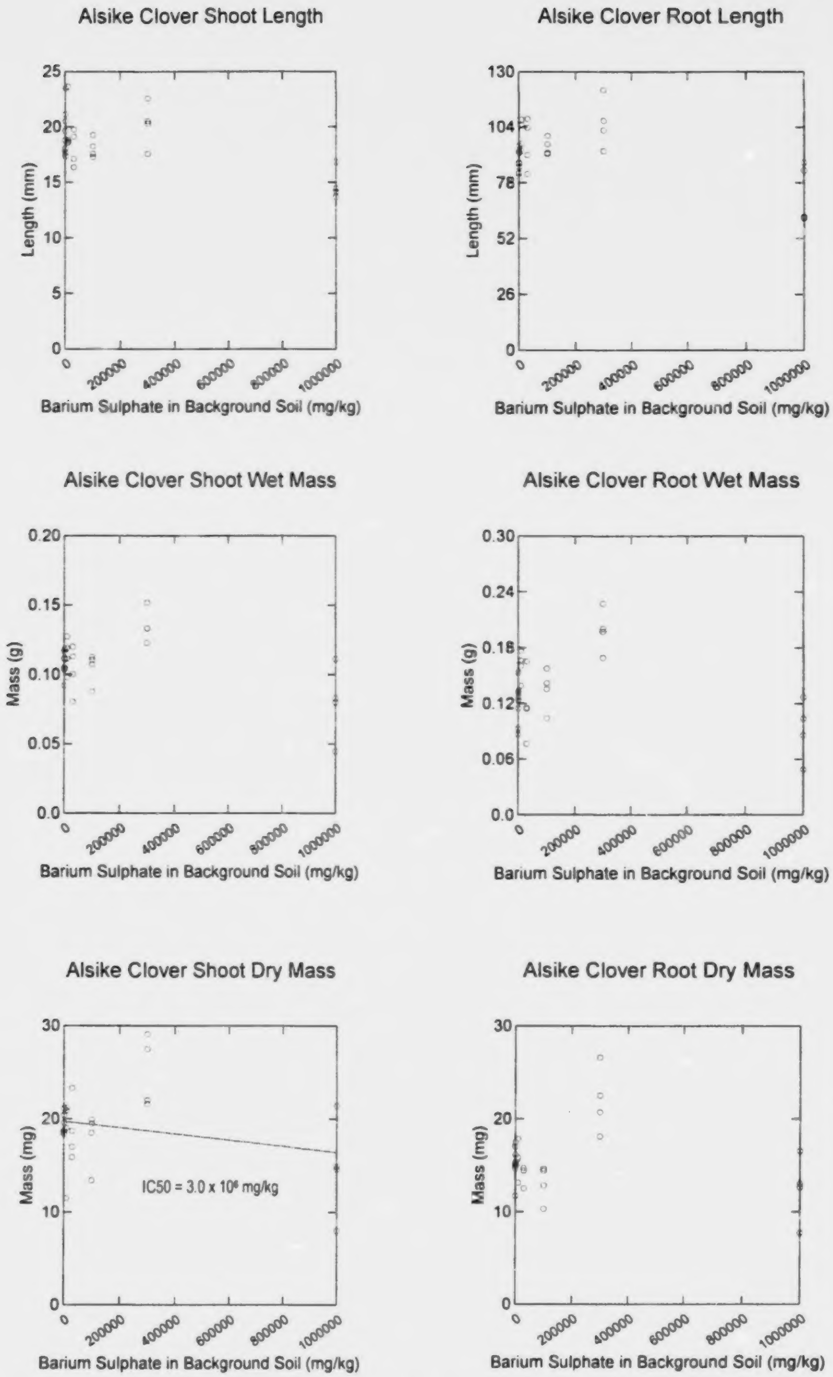


Figure A.3. Emergence of orchardgrass and perennial ryegrass following 14 days, and alsike clover following 21 days of exposure to the experimental control soil (artificial soil, AS), and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars indicate one standard error, and numbers above the columns indicate the number of emerged seedlings per treatment. Asterisks indicate significant differences from the reference control soil treatment.


Alsike Clover Shoot Length

Alsike Clover Root Length

Alsike Clover Shoot Wet Mass

Alsike Clover Root Wet Mass

Alsike Clover Shoot Dry Mass

Alsike Clover Root Dry Mass

Figure A.4 . Alsike clover growth following 21 days of exposure to the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.).

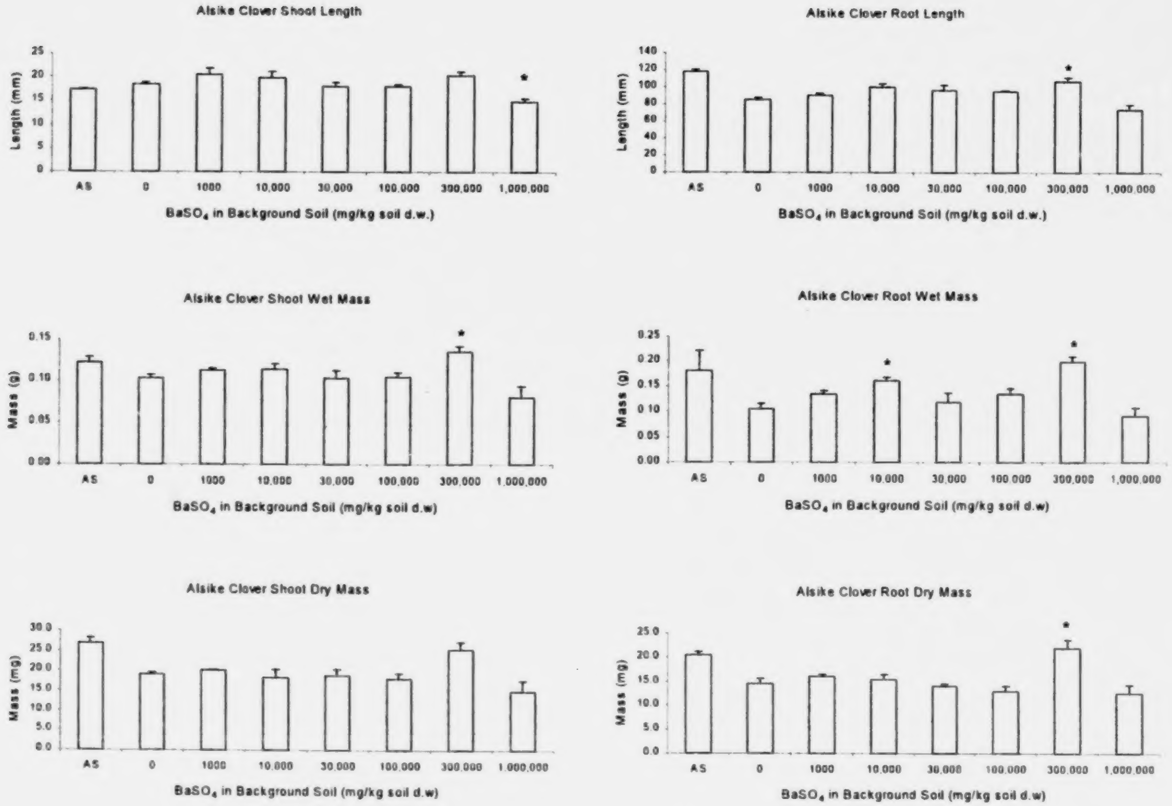


Figure A.5. Alsike clover growth following 21 days of exposure to the experimental control soil (artificial soil, AS), the reference control site soil, and the barium sulphate-amended reference site soils (mg BaSO$_4$/kg soil d.w.). Bars above columns indicate one standard error. Asterisks indicate significant differences from the unamended reference control site soil.

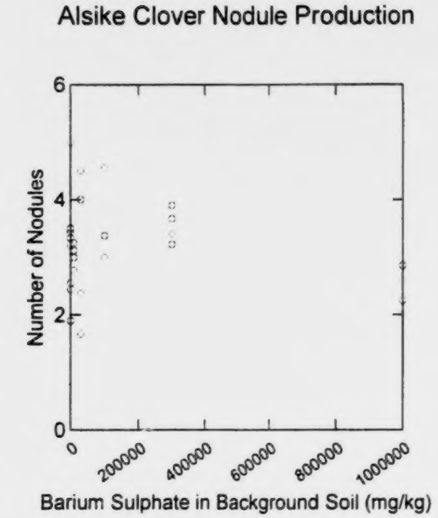


Figure A.6. Nodule production on the roots of alsike clover seedlings following 21 days of exposure to the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.).

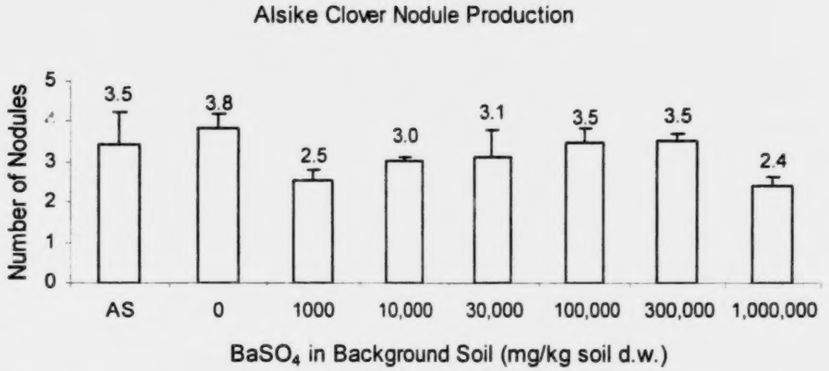


Figure days of exposure to the experimental control soil (artificial soil, AS), reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars above columns indicate one standard error and numbers above the columns indicate the number of nodules observed per treatment.

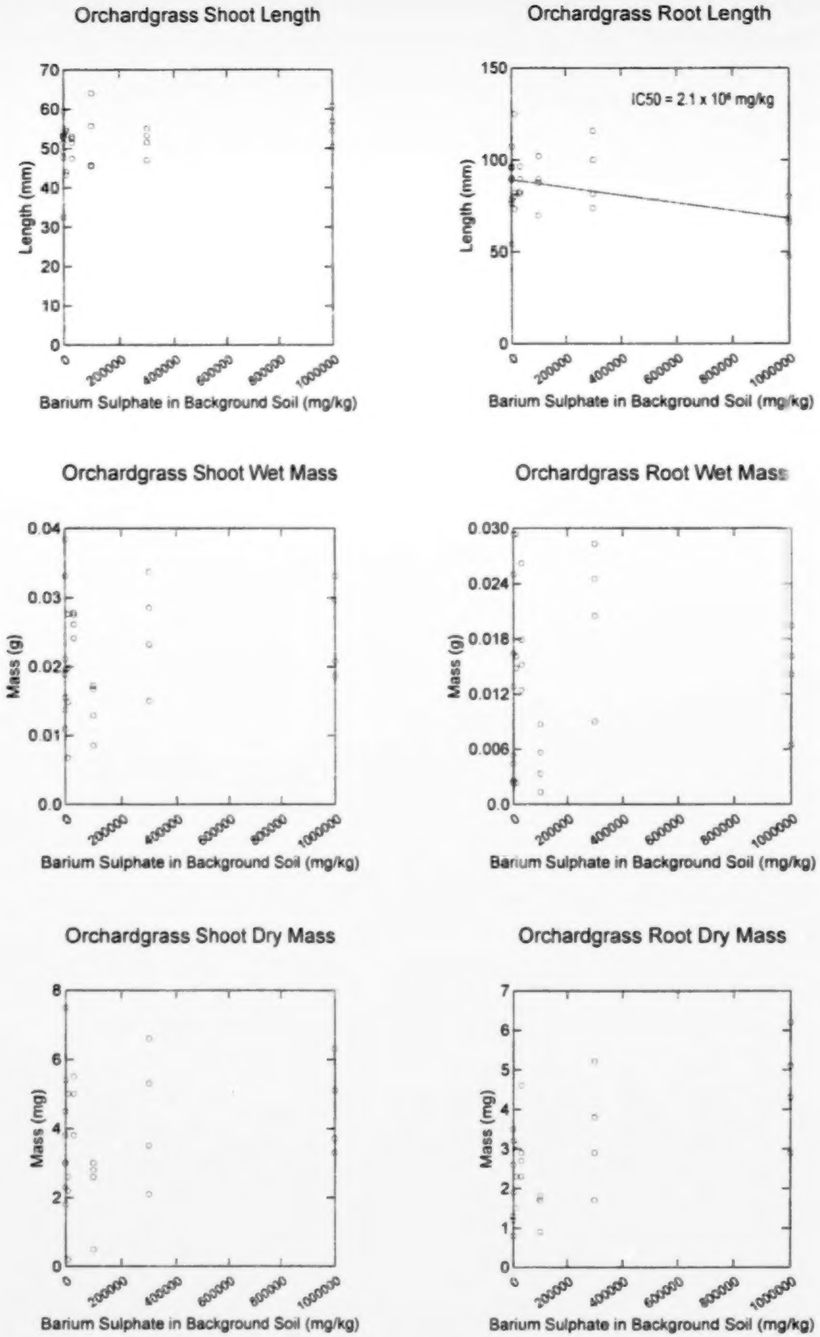


Figure A.8 . Orchardgrass growth following 14 days of exposure to the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.).

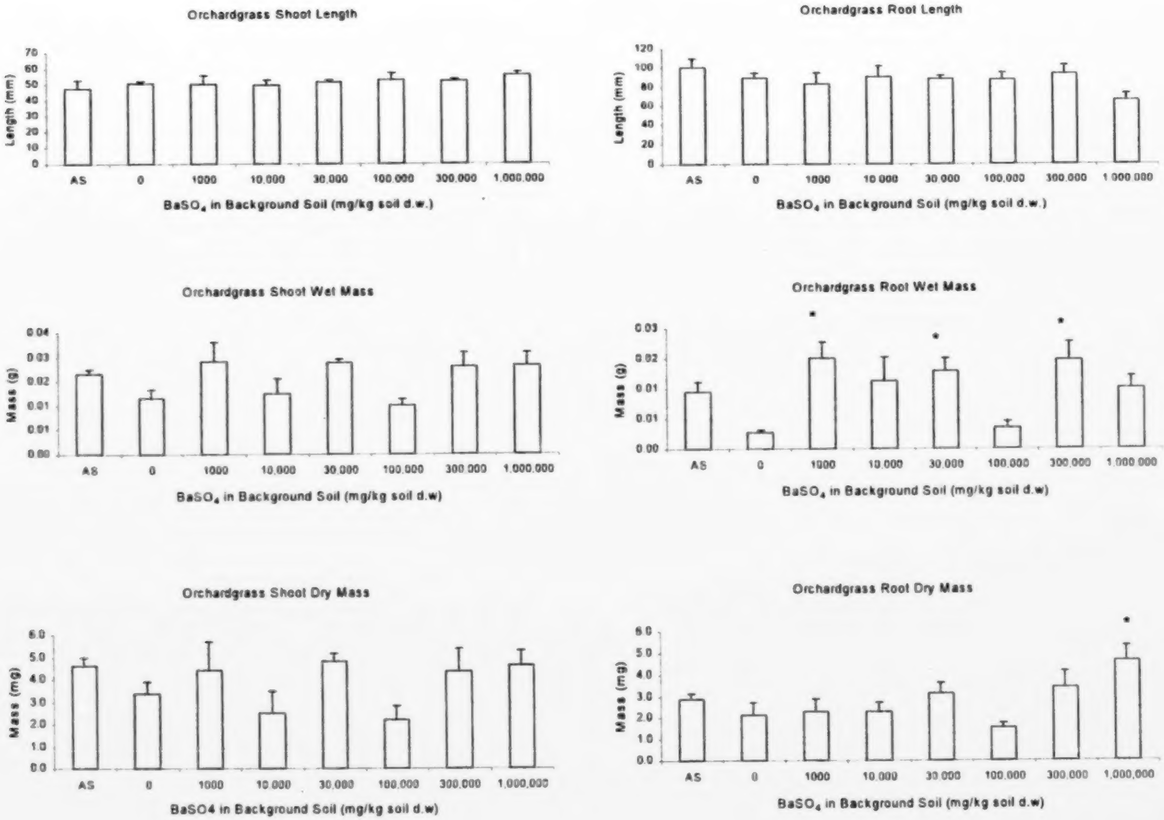


Figure A.9. Orchardgrass growth following 14 days of exposure to the experimental control soil (artificial soil, AS), the reference control site soil, and the barium sulphate-amended reference site soils (mg BaSO$_4$/kg soil d.w.). Bars above columns indicate one standard error. Asterisks indicate significant differences from the unamended reference control site soil.

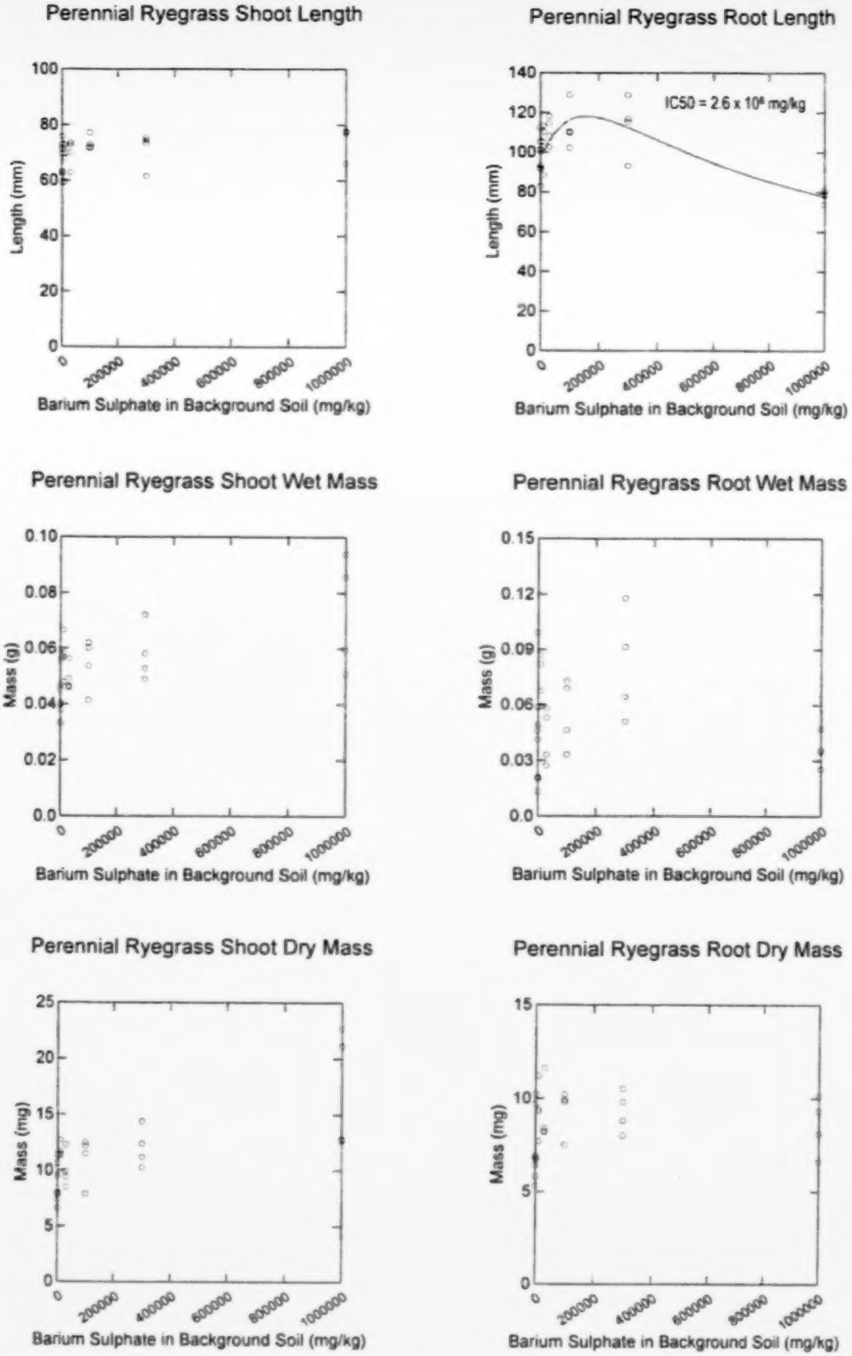


Figure A.10. Perennial ryegrass growth following 14 days of exposure to the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.).

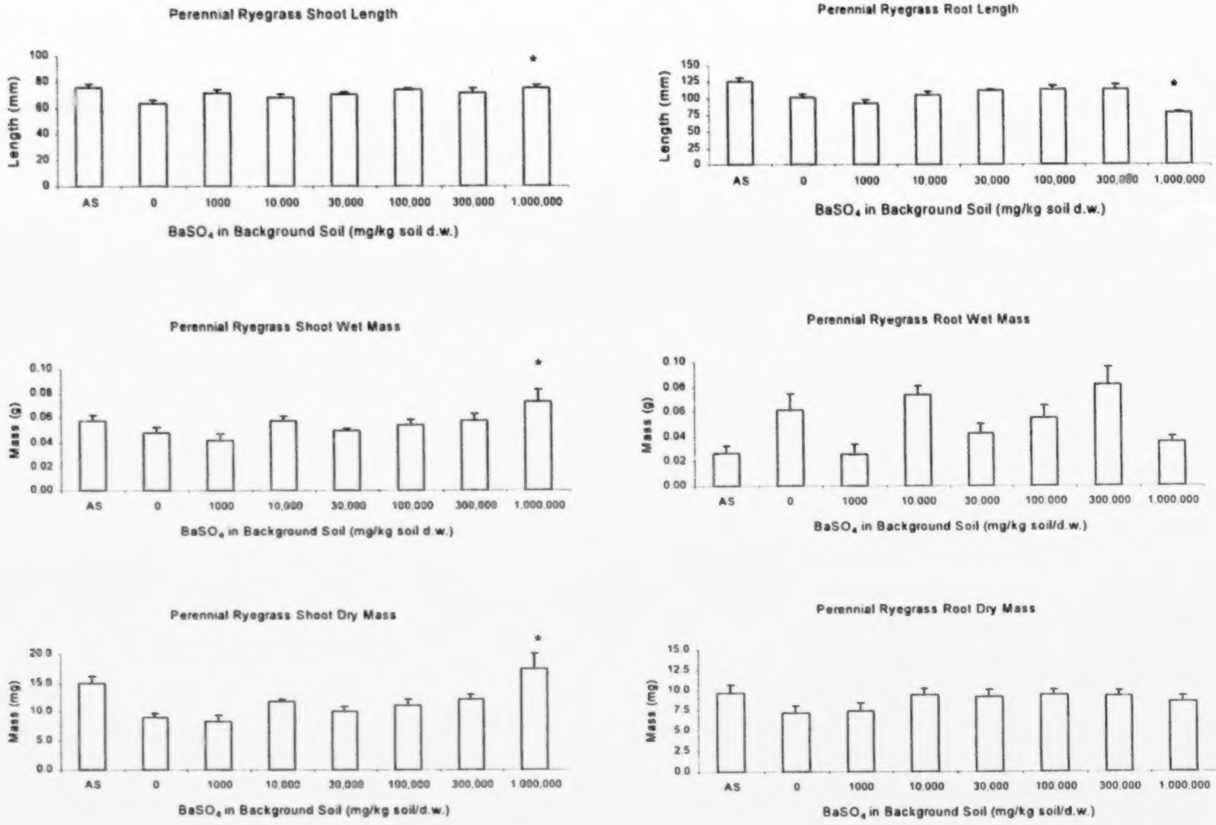


Figure A.11. Perennial ryegrass growth following 14 days of exposure to the experimental control soil (artificial soil, AS), the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars represent one standard error. Asterisks indicate significant differences from the unamended reference control site soil.

Table A.8. Effect of exposure to barium sulphate-amended soil to the growth of alsike clover, orchardgrass and perennial ryegrass. Values in brackets indicate upper and lower confidence limits, respectively.

Species	Parameter	Model	IC50[1] (mg/kg)	IC20[1] (mg/kg)
Alsike clover	Shoot Dry Mass	Linear	> 1,000,000 (-1.2×10^6, 7.1×10^6)	> 1,000,000 (-4.6×10^5, 2.8×10^6)
Orchardgrass	Root Length	Linear	> 1,000,000 (4.0×10^5, 3.9×10^6)	850,000 (1.6×10^5, 1.5×10^6)
Perennial Ryegrass	Root Length	Hormesis	> 1,000,000 (5.7×10^5, 4.6×10^6)	960,000 (6.4×10^5, 1.3×10^6)

[1] Where confidence limits are negative, this indicates that the fit of the model to the data was poor.

Table A.9. The effect of exposure of alsike clover, orchardgrass and perennial ryegrass to barium sulphate-amended reference site soil, reference control soil, and artificial soil (Phase 2 testing). Values in brackets indicate one standard error.

Treatment (mg $BaSO_4$/kg reference soil d.w.)	**Endpoint**					
	Shoot Length (mm)	**Shoot Wet Mass** (g)	**Shoot Dry Mass** (mg)	**Root Length** (mm)	**Root Wet Mass** (g)	**Root Dry Mass** (mg)
Alsike Clover						
Artificial Soil	17.29 (0.35)	0.12 (0.01)	26.78 (1.29)	117.33 (2.66)	0.18 (0.04)	20.45 (0.84)
Reference Soil	18.52 (0.44)	0.10 (0.004)	19.00 (0.47)	85.54 (2.52)	0.11 (0.01)	14.55 (1.09)
1000	20.59 (1.26)	0.11 (0.003)	19.98 (0.50)	90.05 (2.34)	0.13 (0.01)	15.98 (0.52)
10000	19.90 (1.24)	0.11 (0.01)	18.15 (2.27)	100.33 (3.47)	0.16 (0.01)	15.48 (0.97)
30000	18.07 (0.81)	0.10 (0.01)	18.73 (1.63)	96.08 (5.91)	0.12 (0.02)	14.00 (0.05)
100000	18.07 (0.44)	0.10 (0.01)	17.83 (1.50)	94.78 (1.99)	0.13 (0.01)	13.03 (0.99)
300000	20.23 (1.03)	0.14 (0.01)	25.05 (1.91)	105.91 (5.94)	0.20 (0.01)	21.98 (1.79)
1000000	14.76 (0.70)	0.08 (0.01)	14.73 (2.74)	73.64 (6.92)	0.09 (0.02)	12.45 (1.81)
Orchardgrass						
Artificial Soil	47.78 (4.84)	0.023 (0.001)	4.60 (0.38)	100.69 (8.99)	0.013 (0.002)	2.87 (0.26)
Reference Soil	50.36 (1.21)	0.016 (0.002)	3.40 (0.48)	89.41 (4.68)	0.004 (0.001)	2.15 (0.55)
1000	49.70 (5.92)	0.027 (0.005)	4.43 (1.27)	82.43 (11.19)	0.021 (0.004)	2.28 (0.58)
10000	49.16 (3.20)	0.017 (0.004)	2.50 (0.98)	90.06 (11.80)	0.016 (0.006)	2.30 (0.40)
30000	51.08 (1.27)	0.026 (0.001)	4.83 (0.36)	87.58 (3.47)	0.018 (0.003)	3.13 (0.51)
100000	52.71 (4.45)	0.014 (0.002)	2.23 (0.58)	87.17 (6.66)	0.005 (0.002)	1.53 (0.21)
300000	51.69 (1.72)	0.025 (0.004)	4.37 (0.99)	92.71 (9.44)	0.021 (0.004)	3.40 (0.74)
1000000	55.52 (2.17)	0.026 (0.003)	4.60 (0.69)	65.38 (6.72)	0.014 (0.003)	4.63 (0.69)
Perennial Ryegrass						
Artificial Soil	75.05 (2.97)	0.058 (0.004)	15.00 (1.13)	125.45 (5.57)	0.03 (0.01)	9.65 (0.95)
Reference Soil	63.25 (2.21)	0.048 (0.004)	9.00 (0.65)	101.50 (4.14)	0.06 (0.01)	7.07 (0.94)
1000	70.74 (2.79)	0.042 (0.005)	8.33 (1.06)	93.20 (4.63)	0.03 (0.01)	7.37 (0.97)
10000	67.45 (2.85)	0.057 (0.004)	11.78 (0.32)	104.20 (5.53)	0.07 (0.01)	9.40 (0.72)
30000	69.80 (2.46)	0.049 (0.002)	10.03 (0.81)	110.80 (3.40)	0.04 (0.01)	9.10 (0.83)
100000	73.49 (1.27)	0.054 (0.005)	11.03 (1.06)	112.83 (5.65)	0.06 (0.01)	9.35 (0.62)
300000	71.16 (3.22)	0.058 (0.005)	12.08 (0.89)	113.49 (7.41)	0.08 (0.01)	9.27 (0.55)
1000000	74.63 (2.79)	0.073 (0.010)	17.33 (2.66)	78.03 (1.51)	0.04 (0.004)	8.52 (0.76)

BARITE SOIL GUIDELINES PROJECT

File: 626 03291

Prepared by:

Stantec Consulting Ltd.
361 Southgate Drive
Guelph ON N1G 3M5

Prepared for:

Barite Soil Guidelines Technical Steering Committee

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Appendix VII

Stantec (2003) –
Barium Acetate Ecotoxicity

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APPENDICES

APPENDIX A RESULTS OF THE SITE SOIL ASSESSMENT: ECOTOXICITY EVALUATION WITH PLANT AND INVERTEBRATE SPECIES

1 Introduction

In March 2003, ESG International (now Stantec Consulting Ltd.) was contracted by Chevron Canada Resource to provide an ecotoxicity assessment of site soils contaminated with a mixture of petroleum hydrocarbons and barium sulphate. The soils originated from the drilled and abandoned Chevron Canada Resources CDN SUP Waterton 04-13-05-01 W5M wellsite, located near Pincher Creek, Alberta. The results of the acute earthworm test indicated that the contaminated site soil was not acutely toxic to earthworms; however, there was significant reduction in growth of two of the three plant species tested, following longer-term exposure to the contaminated soil. Petroleum hydrocarbon soil concentrations in the contaminated soil tested were not at levels expected to have caused the adverse effects. The barium sulphate soil concentration, as total barium sulphate, in the contaminated soil was at levels sufficiently high to potentially cause adverse effects; however, the level of barium sulphate as saturated paste, which represents the water soluble fraction, was extremely low. As a result, Chevron Canada, on behalf of the Canadian Association of Petroleum Producers (CAPP), contracted ESG to provide a preliminary ecotoxicity assessment of the previously-collected reference control site soil amended with barium acetate. Barium acetate was chosen as the toxicant because it is more soluble than barium sulphate and more representative of a worse-case exposure scenario. The reference control site soil is a fine-grained Alberta till (very clayey) soil that was collected by collected by Komex International Inc. from a site in Alberta. The physical and chemical characteristics of the reference control soil were similar to that of the contaminated site soil, but the soil was free of contamination.

The preliminary toxicity assessment included three experiments: an acute earthworm toxicity test, an acute collembola toxicity test and an acute plant screening test. The experiments were conducted with reference control site soil (e.g., background soil) amended with different quantities of industrial-grade barium acetate, as well as with an experimental control soil (e.g., artificial soil). The worm and plant test species were identical to those used in the previous testing program with the contaminated site soil; acute tests were also conducted with a soil arthropod (a collembola, or springtail, species). The test species included the earthworm *Eisenia andrei* (compost worm), the collembola *Onychiurus folsomi* and the leguminous plant, alsike clover (*Trifolium hybridum*). These species had been originally chosen because *E. andrei* and collembola are commonly used to assess the effects of contaminated soil on terrestrial invertebrates, and alsike clover is used as a forage crop on rangeland in Alberta. Following the preliminary toxicity assessment, chronic tests were performed to assess the effects of longer exposure durations. In addition to those test species listed above, tests with perennial ryegrass (*Lolium perenne*) and orchard grass (*Dactylis glomerata*) were also conducted.

The reference control soils (Background-2) were collected by Komex International and received by ESG on February 18 and March 14, 2003. After their arrival at ESG's Southgate Laboratory, the soil samples (e.g., 20-L buckets) were assigned identification numbers and were entered into a logbook (Table A.1; Appendix A). Five buckets of the background soil were received, and the samples were denoted as Background-2 (1-5). Prior to testing, the soil samples were air-dried for 24 hours, broken up by hand, pulverized into smaller aggregates, and sieved through a 6-mm stainless steel sieve. Once all the soil from the five buckets had been sieved, they were homogenized and stored in plastic 20-L buckets at room temperature (20 ± 2°C) until use. The soil moisture content of the background and artificial soils was determined prior to testing.

The experimental control soil was an artificial soil (AS) formulated in the laboratory, and characterized as a fine sandy loam. The physical and chemical characteristics of the experimental control soil are listed in Table A.2 (Appendix A).

The objective of this research was to determine the concentration of barium acetate in the reference control site soil (Background-2 soil) that would adversely affect earthworm and collembola survival and seedling emergence and early growth. The experimental control soil provided QA/QC data on test organism health and experimental conditions. Testing began on May 14, 2003. The results of the testing are summarized and presented in this report .

2 Chemical Analysis

Barium soil concentration of the $Ba(C_2H_3O_2)_2$-amended soils was measured at the beginning (Day –1 for earthworms, Day 0 for plants) and at the end (Day 14 for earthworms, Day 21 for plants) of plant and earthworm tests. Soil subsamples were collected from each amended treatment at the beginning and end of the *Eisenia andrei* acute tests and the beginning and end of the *Eisenia andrei* chronic and alsike clover definitive tests. Samples were collected from the two different species to observe the effect, if any, of the presence of earthworms *vs.* plants on the total or water-soluble concentration of barium in the soil. Barium acetate was analysed as total soil concentration and as saturated paste. Barium acetate is soluble and, as a result, the $Ba(C_2H_3O_2)_2$-saturated paste values were expected to represent the water-soluble fraction of barium in the soil. The chemical analyses were performed by Philip Analytical Services Corporation, Mississauga, Ontario.

The total barium and barium as saturated paste concentrations in the soil samples collected from the *Eisenia andrei* (acute and definitive) and alsike clover (definitive) tests are presented in Table A.3 (Appendix A). There was no discernable effect of test species (i.e., plant vs. earthworm) on any of the measured soil concentrations between the *E. andrei* and alsike clover end-of-test results.

For all three sets of analyses, the reference control soil (0 mg/kg) had relatively high background total barium concentrations that ranged from 239 to 268 mg/kg soil d.w.. The measured concentrations are in good agreement with the nominal concentrations once background barium concentrations are accounted for, since 255.43 g (molecular weight) barium acetate is equivalent to 137.33 g (molecular weight) of barium. Nominal barium soil concentrations were regressed against measured barium concentrations in soil analyzed from the beginning and end of the earthworm acute and plant definitive tests, and from the beginning of the earthworm chronic test. The measured values of total barium were corrected for background barium concentrations, and the results show an increasing concentration gradient that was strongly correlated with nominal barium exposure concentrations (Figures A.1 to A.3; Appendix A). Regression co-efficients (r^2 values) for all sampling times were equal to or greater than 0.999.

The concentration of barium measured as saturated paste did not correspond well to either the nominal barium soil concentrations or the measured total barium concentrations for all three sets of analyses. A concentration gradient existed among soil treatments sampled at the beginning (Day –1) of the acute earthworm test (Table A.3; Appendix A) but not in soils sampled at the end (Day 14) of the test. No obvious patterns or trends were discerned among treatments of the soils sampled at the beginning or end of the definitive plant test, nor in soil sampled at the beginning of the chronic earthworm test (Table A.3; Appendix A).

Samples of soil treatments were only analyzed from the beginning of the earthworm chronic test. No samples were collected on the last day of the test. Based on the results shown in Table A.3 (Appendix A), it was decided that the saturated paste extraction of barium was not a good surrogate for the water-soluble, or bioavailable, fraction of barium in soil.

3 Acute Toxicity To *Eisenia andrei*

3.1 EXPERIMENTAL DESIGN AND CONDITIONS

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- 14-day duration;
- Test endpoint: adult survivorship
- Temperature: constant at 20 ± 2°C; and,
- Photoperiod: 16-h day, 8-h night (fluorescent illumination).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background-2") soil, and batches of reference ("Background-2") soil amended with sufficient barium acetate to achieve the following concentration series: 2000, 4000, 6000, 8000, 10,000, and 12,000 mg $Ba(C_2H_3O_2)_2$/kg soil d.w.

Test units consisted of 500-mL, wide-mouthed glass mason jars filled with 270 g soil wet weight (w.w.) of test soil, for both the AS and Background-2 reference soils. Jars were covered with perforated tin foil held by a metal screw ring to facilitate gas exchange for the earthworms. Each treatment included 3 replicate test units. Five adult earthworms were added to each test unit. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998a).

3.2 SOIL PREPARATION

On Day -1, the control and amended soils were prepared on a dry weight basis. Barium acetate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting $Ba(C_2H_3O_2)_2$-soil mixture (referred to as BaAc) was then hydrated with reverse osmosis (R.O.) water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the soils ranged from 17 to 35%. Physical and chemical soil parameters such as pH, electrical conductivity, and moisture content were measured following test soil preparation and at the end of the test (Table A.4; Appendix A).

On Day 0, five clitellate *Eisenia andrei* were added to each test unit and the soils in the test units were misted gently with reverse osmosis (R.O.) water as necessary.

Substantially more water was added to the test units of the reference soil treatments after the earthworms were added to the test units, as compared to the experimental control soil treatment. The extremely clayey nature of the site soils precluded the addition of the required volume of R.O. water while homogenizing the soil; therefore, most of the water was added immediately after the earthworms were added.

3.3 STATISTICAL ANALYSES

Acute earthworm test results were analyzed using the Trimmed Spearman-Karber regression analysis (Stephan, 1989) to generate 7- and 14-d LC50 estimates and their 95% confidence limits.

3.4 RESULTS

On Day 7, each test unit was inspected to determine the number and condition of surviving *E. andrei*. The 7-d LC50 was 7673.4 mg BaAc/kg soil d.w. and the associated 95% confidence limits were 7193.3 and 8185.6 mg/kg soil d.w.. The 14-d LC50 was 7168.2 mg BaAc/kg soil d.w. with 95% confidence limits of 6853.9 and 7496.9 mg/kg soil d.w. (Table A.5; Appendix A). All earthworms in each treatment up to and including 6000 mg/kg BaAc soil d.w. were alive. In the 8000 mg/kg BaAc soil d.w. treatment, only 6 worms remained alive (n=15); and all surviving worms appeared unhealthy. All the worms in 10,000 and 12,000 mg/kg treatments were dead after 7 days. (Table A.5; Appendix A).

On Day 14, each test unit was inspected again to determine the number and condition of surviving *E. andrei*. All earthworms in each treatment up to and including 6000 mg/kg BaAc soil d.w. were still alive; however, most worms had re-sorbed their clitellums, a sign of stress. In the 8000 mg/kg BaAc soil d.w. treatment, only 2 worms remained alive, and appeared to be unhealthy with re-sorbtion of their clitellum, pinching-off in the midsection of their body and lethargic movement (Table A.5; Appendix A). Soil pH, electrical conductivity and soil moisture content were measured and compared among the experimental control, reference control (0 mg/kg BaAc soil d.w.), and BaAc-amended reference soils. The addition of barium acetate to the reference soil did not affect soil pH, and all soil pH values were within a range acceptable for *E. andrei* survival and growth. Soil moisture content (end of test) was comparable among all treatments, and was very similar among the reference soil treatments. Soil electrical conductivity in the reference soil increased because of the addition of barium acetate, and reached 2050 µS/cm at the highest concentrations, (Table A.4; Appendix A) which is outside of the range considered acceptable for *E. andrei* survival and growth (earthworm growth has been observed to be unaffected up to 1500 µS/cm).

4 Acute Toxicity To *Onychiurus folsomi*

4.1 EXPERIMENTAL DESIGN AND CONDITIONS

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- 7-day duration;
- Test endpoint: adult survivorship
- Temperature: constant at 20 ± 2°C; and,
- Photoperiod: 16-h day, 8-h night (fluorescent illumination).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background-2") soil, and batches of reference ("Background-2") soil amended with sufficient barium acetate to achieve the following concentration series: 2000, 4000, 6000, 8000, 10,000, and 12,000 mg $Ba(C_2H_3O_2)_2$/kg soil d.w.

Test units consisted of 125-mL, wide-mouthed glass mason jars filled with 30 g soil wet weight (w.w.) of test soil, for both the AS and Background-2 reference soils. Jars were covered with metal lids screwed on very lightly by a metal screw ring to facilitate gas exchange. Each treatment included 3 replicate test units. Ten (10) adult collembola were added to each test unit. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998c).

4.2 SOIL PREPARATION

On Day -1, the control and amended soils were prepared on a dry weight basis. Barium acetate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting BaAc-soil mixture was then hydrated with R.O. water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the soils ranged from 17 to 35%. Physical and chemical soil parameters such as pH, electrical conductivity, and moisture content were measured following test soil preparation and at the end of the test (Table A.4; Appendix A).

On Day 0, 10 adult *Onychiurus folsomi* were added to each test unit and the soils in the test units were misted gently with R.O. water as necessary. Substantially more water was added to the test units of the reference soil treatments after the collembola were added to the test units, as compared to the experimental control soil treatment. The extremely clayey nature of the site soils precluded the addition of the required volume of R.O. water while homogenizing the soil, therefore most of the water was added immediately after the collembola were added.

4.3 STATISTICAL ANALYSES

No statistical analyses of the collembola toxicity data were conducted. The data sets and their individual histograms provided a visual estimate of the LC50.

4.4 RESULTS

On Day 7, each test unit was inspected to determine the number and condition of surviving *O. folsomi*. No apparent difference in mortality in any of the treatments following 7 days of exposure, and all surviving collembola appeared healthy (Table A.6; Appendix A). Soil pH, electrical conductivity and soil moisture content were measured and compared among the experimental control, reference control (0 mg/kg BaAc soil d.w.), and BaAc-amended reference soils. The addition of barium acetate to the reference soil did not affect soil pH, and all soil pH values were within a range acceptable for *O. folsomi* survival and growth. Soil moisture content (end of test) was comparable among all treatments, and was very similar among the reference soil treatments. Soil electrical conductivity in the reference soil was increased by the addition of barium acetate, reaching a value of 2050 µS/cm (Table A.4; Appendix A).

5 Preliminary Screening Plant test

5.1 EXPERIMENTAL DESIGN AND CONDITIONS

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- Test organism was alsike clover from Pickseed Canada Inc., Sherwood Park, AB;
- Test duration: 14 d;
- Temperature: 24/16 ± 2°C day/night;
- Photoperiod: 16/8-h day/night ;
- Light intensity: ~ 300-350 ΦE/m^2/sec;

Test endpoint: seedling emergence, shoot and root wet and dry mass and number of nodules.

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background-2") soil, and batches of reference ("Background-2") soil amended with sufficient barium acetate to achieve the following concentration series; 500, 1000, 2000, 4000, 6000, 8000, 10,000 and 12,000 mg BaAc/kg soil d.w.

Test units consisted of a glass petri dish enclosed within an inverted 1-L clear polypropylene container. Each petri dish was filled with 100 g w.w. of AS at 35% moisture content or 100 g w.w. of reference ("Background-2") soil at 17% moisture content. Both AS and reference soil treatments consisted of 2 replicate test units. Ten alsike clover seeds were planted in each test unit. Methods and procedures for site soil amendment are described in detail in EC, 1998b.

5.2 SOIL PREPARATION

On Day 0, the control and amended soils were prepared on a dry weight basis, but volumes were adjusted to compensate for differences in bulk density of the soils. Barium acetate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting BaAc-soil mixture was then hydrated with R.O. water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the prepared test soils ranged from 17 to 35 %. In addition, the extremely clayey nature of the background soil precluded the addition of the optimal volume of R.O. water while homogenizing the soil, therefore most of the water was added immediately after the seeds were planted. Physical and chemical soil parameters such as pH and electrical conductivity were measured following test soil preparation and at the end of the test (Table A.4; Appendix A). Seeds were planted in each test unit, to a depth of approximately the length of the longest side of the seed. Test units were then misted gently with R.O. water. Substantially more water was added to the test units containing the clay background soil as compared to the experimental control soil. Test units were checked daily and watered on an as-need basis with well-aerated de-chlorinated, municipal tap water.

5.3 TEST PROCESSING

On Day 14, each test unit was inspected to determine the number and condition of emerged seedlings. A seedling was considered emerged if the shoot tip was greater than 3 mm above the surface of the soil. Seedlings were carefully removed from the soil and any soil particles adhering to the roots were gently washed from each seedling using a spray bottle. Once cleaned, seedling shoot and root length were measured as well as shoot and root wet mass. Plant material was dried for 48 hours at 90°C after which shoot and root dry mass was also determined. The roots of alsike clover plants were inspected after length measurements were taken, and any obvious effect on the number of nodules present per plant, per treatment, was recorded.

5.4 STATISTICAL ANALYSES

No statistical analyses of the alsike clover toxicity data were conducted. The data histograms provided a visual estimate of the EC50s.

5.5 RESULTS

Alsike clover emergence in the reference soil was adversely affected at the 2000 mg BaAc/kg soil d.w. treatment and greater (Figure A.4; Appendix A).

In general, seedling growth was adversely affected at the 1000 mg BaAc/kg soil d.w. treatment and greater compared to the reference control soil (0 mg BaAc /kg soil d.w.) (Table A.7, Figure A.5; Appendix A). Adverse effects on shoot length began at 500 mg/kg soil d.w., while shoot dry mass and number of nodules were affected at 2000 mg/kg soil d.w. and greater.

Soil pH and electrical conductivity were measured and compared among the test soils, and the values for both parameters in all soil treatments were within the range that supports plant growth. The addition of barium acetate to the reference soil did not affect pH or conductivity (Table A.4; Appendix A).

6 Definitive Plant Toxicity Tests

6.1 EXPERIMENTAL DESIGN AND CONDITIONS

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- Test organisms were alsike clover, perennial ryegrass and orchard grass from Pickseed Canada Inc., Sherwood Park, AB;
- Test duration: 14 d for perennial ryegrass and orchard grass; 21 d for alsike clover
- Temperature: 24/16 ± 2°C day/night;
- Photoperiod: 16/8-h day/night ;
- Light intensity: ~ 300-350 ΦE/m^2/sec;

Test endpoint: seedling emergence, shoot and root wet and dry mass (phytotoxicity and number of nodules for alsike clover)

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background-2") soil, and batches of reference ("Background-2") soil amended with sufficient barium acetate to achieve the following concentration series: 75, 150, 300, 500, 1000, and 2000 mg BaAc/kg soil d.w.

Test units consisted of a 1-L clear polypropylene container filled with 485 g w.w. of AS at 35% moisture content or 375 g w.w. of reference ("Background-2") soil at 17% moisture content. Both the AS and reference soil control treatments consisted of 6 replicate test units. The 75, 150, and 300 mg BaAc/kg soil d.w. treatments each had 4 replicate test units, whereas the 500 1000 and 2000 mg BaAc/kg soil d.w. treatments each had 3 replicate test units.

Ten alsike clover seeds were planted in each test unit, while orchard grass and perennial ryegrass both had five seeds per test unit. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998b).

6.2 SOIL PREPARATION

On Day 0, the control and amended soils were prepared on a dry weight basis. Barium acetate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting BaAc-soil mixture was then hydrated with R.O. water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the prepared test soils ranged from 17 to 35 %. In addition, the extremely clayey nature of the background soil precluded the addition of the optimal volume of R.O. water while homogenizing the soil, therefore most of the water was added immediately after the seeds were planted. Physical and chemical soil parameters such as pH and electrical conductivity were measured following test soil preparation and at the end of the test (Table A.4; Appendix A). Seeds were planted in each test unit, to a depth of approximately the length of the longest side of the seed. Test units were then misted gently with R.O. water. Substantially more water was added to the test units containing the clayey background soil as compared to the experimental control soil. Test units were checked daily and watered on an as-need basis with well-aerated de-chlorinated, municipal tap water.

6.3 TEST PROCESSING

On the final testing day (Day 14 for orchard grass and perennial ryegrass; Day 21 for alsike clover) each test unit was inspected to determine the number and condition of emerged seedlings. A seedling was considered emerged if the shoot tip was greater than 3 mm above the surface of the soil. Seedlings were carefully removed from the soil and any soil particles adhering to the roots were gently washed from each seedling using a spray bottle. Once cleaned, seedling shoot and root length were measured as well as shoot and root wet mass. For alsike clover, two more endpoints were measured: phytotoxicity and number of nodules. The roots of alsike clover plants were inspected after length measurements were taken, and any obvious effect on the number of nodules present per plant, per treatment was recorded. Plant material was dried for 48 hours at 90°C after which shoot and root dry mass was also determined.

6.4 STATISTICAL ANALYSES

Analyses of variance (ANOVA) procedures were applied to the data and a two-tailed Dunnett's test was used to compare each treatment mean to the mean of the reference (unamended Background-2 soil) control soil treatment. The Dunnett's pairwise comparison test was used to compare treatment means of the amended reference soils to the control reference soil to determine the no-observable-adverse-effect concentrations (NOAEC) and lowest-observable-adverse-effect concentrations (LOAEC) (SPSS, 1997). Fisher's Least Significant Difference Test, using pairwise comparison probabilities, was used to determine significant differences among treatment means (SPSS, 1997). Linear or non-linear regression procedures were also applied to the data in order to generate IC50 and IC20 estimates. The regression analyses consisted of using a linear or four nonlinear regression models (i.e., logistic, gompertz, exponential and logistic with hormesis) that had been re-parameterized to include the ICp and the associated 95% confidence limits. The ICp is the inhibition concentration (IC) resulting in a specified percentage (p) effect. The data were examined for normality and homogeneity of variances among treatments. All analyses were performed with SYSTAT 7.0.1 (SPSS, 1997). A more detailed description of the statistical procedures used to analyze the definitive plant toxicity test data can be found in either Environment Canada (1998b) or Stephenson *et al.* (2000).

6.5 RESULTS

6.5.1 Alsike Clover

Emergence in the control soils was 85%. Emergence in the amended soils was adversely affected relative to that in the control soil at the treatment concentration of 1000 mg BaAc/kg soil d.w.) (Figure A.6; Appendix A). In general, number of nodules, and shoot- and root -length were the least sensitive endpoints for alsike clover. Root wet- and dry mass were the most sensitive endpoints (Figures A.7 to A.9; Appendix A). The ICp values (IC20, IC50) and their associated 95% confidence limits, and NOAEC and LOAEC estimates for alsike clover are summarized in Tables A.8a and b (Appendix A).

6.5.2 Orchard grass

Emergence in the control soil was 70%. Emergence in the amended soils was adversely affected relative to that in the control soil at the treatment concentration of 1000 mg BaAc/kg soil d.w.) (Figure A.6; Appendix A). The least sensitive endpoints for orchard grass were shoot and root length, followed by shoot dry mass and root wet mass. Shoot wet mass and root dry mass were the most sensitive endpoints (Figures A.10 and A.11; Appendix A). The ICp values (IC20, IC50) and their associated 95% confidence limits, and NOAEC and LOAEC estimates for orchard grass are summarized in Tables A.9a and b (Appendix A).

6.5.3 Perennial ryegrass

Emergence in the control soils was 87%. Emergence in the amended soils was adversely affected, but not significantly, relative to that in the control soil at the treatment concentration of 2000 mg BaAc/kg soil d.w. (Figure A.6; Appendix A). In general, shoot and root length were the least sensitive endpoints. Shoot wet and dry mass were the most sensitive endpoints (Figures A.12 and A.13; Appendix A). The ICp values (IC20, IC50) and their associated 95% confidence limits, and NOAEC and

LOAEC estimates for orchard grass are summarized in Tables A.10a and b (Appendix A).

For all three plant tests, soil pH and electrical conductivity were measured and compared among the test soils. The addition of BaAc to the reference soil affected both pH and conductivity; however, changes in both these parameters were well within the range that supports healthy plant growth (Table A.4; Appendix A).

7 Definitive Invertebrate Toxicity Tests

7.1 EXPERIMENTAL DESIGN AND CONDITIONS

The experimental design and conditions of the test were as follows:

- Test type was static chronic;
- Test organisms were *Eisenia andrei* and *Onychiurus folsomi* from in-house cultures;
- Test duration: 35 d for *O. folsomi*; 63 d for *E. andrei*;
- Temperature constant at 20 ± 2°C day/night;
- Photoperiod: 16/8-h day/night (fluorescent illumination); and,
- Test endpoints: adult survivorship, adult fecundity, number of juveniles

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background-2") soil, and batches of reference ("Background-2") soil amended with sufficient barium acetate to achieve the following concentration series for *E. andrei*: 10, 30, 60, 100, 300, 600, 1000, 3000, 6000 mg BaAc/kg soil; and 100, 300, 600, 1000, 3000, 6000, 8000, 10 000, and 12,000 mg BaAc/kg soil d.w. for *O. folsomi*.

E. andrei test units consisted of 500-mL, wide-mouthed glass mason jars filled with 270 g soil wet weight (w.w.) of all test soils. Jars were covered with perforated tin foil held by a metal screw ring to facilitate gas exchange for the earthworms. Each treatment included 10 replicate test units. Two adult worms were added to each test unit.

O. folsomi test units consisted of 125-mL, wide-mouthed glass mason jars filled with 30 g soil wet weight (w.w.) for all test soils. Jars were covered with metal lids screwed on very lightly by a metal screw ring to facilitate gas exchange. Each treatment included 10 replicate test units. Ten (10) adult collembola were added to each test unit.

Methods and procedures for site soil amendment are elsewhere (EC, 1998a and c).

7.2 SOIL PREPARATION

On Day 0, the control and amended soils were prepared on a dry weight basis. Barium acetate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting BaAc-soil mixture was then hydrated with R.O. water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the prepared test soils ranged from 17 to 35 %. In addition, the extremely clayey nature of the background soil precluded the addition of the optimal volume of R.O. water while homogenizing the soil, therefore most of the water was added immediately after the organisms were added by gentle misting with R.O. water. Substantially more water was added to the test units containing the clay background soil as compared to the experimental control soil. Test units were checked, watered with R.O. and fed with oatmeal on a weekly basis. Physical and chemical soil parameters such as pH and electrical conductivity were measured following test soil preparation and at the end of the test (Table A.4; Appendix A).

7.3 TEST PROCESSING

On the final testing day for *O. folsomi*, (Day 35) each test unit was inspected to determine the condition and surviving number of adult collembola, along with number of juveniles produced. An adult was considered alive if it showed signs of movement within a 1-2 minute period. If a juvenile collembolan showed no immediate sign of movement, it was not enumerated. The collembola were floated to the top of each test unit by pouring water into the test unit until the soil surface was covered, then carefully removed with a very fine paintbrush and placed into a petri dish to be enumerated.

E. andrei adults were removed on Day 35 and the number of surviving adults were recorded. On day 63 the test was processed to determine the number of juveniles produced. Juvenile wet and dry mass were also measured, after the juveniles were dried for 48 hours at 90°C

7.4 STATISTICAL ANALYSES

O. folsomi: Analyses of variance procedures were applied to the data and a two-tailed Dunnett's test was used to compare each treatment mean to the mean of the reference (unamended, Background-2) control soil treatment. The Dunnett's pairwise comparison test was used to compare treatment means of the amended reference soils to the control reference soil to determine the no-observable-adverse-effect concentrations (NOAEC) and lowest-observable-adverse-effect concentrations (LOAEC) (SPSS, 1997). Fisher's Least Significant Difference Test, using pairwise comparison probabilities, was used to determine where the significant differences among treatment means occurred (SPSS, 1997). Linear or non-linear regression procedures were also applied to the data in order to generate IC50 and IC20 estimates. The regression analyses consisted of using a linear or four nonlinear regression models (i.e., logistic, gompertz, exponential and logistic with hormesis) that had been re-parameterized to include the ICp and the associated 95% confidence limits. The ICp is the inhibition concentration (IC) resulting in a specified percentage (p) effect. The data were examined for normality and homogeneity of variances among treatments. All analyses were performed with SYSTAT 7.0.1 (SPSS, 1997). A more detailed description of the statistical procedures used to analyze the definitive plant toxicity test data can be found in either Environment Canada (1998) or Stephenson *et al.* (2000).

E. andrei: no statistical analysis was performed on the definitive *E. andrei* test due to deficiencies in the data set (see Subsection 7.5.2).

7.5 RESULTS

7.5.1 *O. folsomi*

O. folsomi adults survived in the highest treatment of 12,000 mg/kg BaAc soil d.w.; however, the number of surviving adults started to decline significantly in the 6000 mg/kg treatment and greater. Juvenile production was adversely affected at 1000 mg/kg BaAc soil d.w., and there were no juveniles at the highest treatment level of 12, 000 mg/kg BaAc soil d.w. Fecundity was adversely affected at 1000 mg/kg BaAc soil d.w. and greater (Figures A.14 and A.15; Appendix A). Estimates of toxicity (EC20, EC50, NOAEC and LOAEC) are summarized in Tables A.11a and b (Appendix A).

7.5.2 *E. andrei*

Adults showed a slight decline in survival in the highest treatment of 6000 mg BaAc/kg soil d.w. Both juvenile production and adult fecundity decreased relative to the control (Figure A.16; Appendix A) at 1000 mg BaAc/kg soil d.w. and was absent at 3000 mg BaAc/kg soil d.w. In the experimental control soil (AS) the mean number of juveniles produced was 23.5. This meets the Environment Canada draft criteria for test acceptability (EC, 2002). In the unamended reference control soil ("Background–2") the mean number of juveniles produced was 1.2. Adult fecundity was also very low in the reference control soil. The low numbers of juveniles and fecundity in both the amended and control site soil indicates that this soil provides an unsuitable habitat for *E. andrei* reproduction.

For the two invertebrate tests, soil pH and electrical conductivity were measured and compared among the test soils. The addition of BaAc to the reference soil affected both pH and conductivity in both invertebrate tests; however, changes in both these parameters were well within values that support *O. folsomi* and *E. andrei* reproduction and survival (Table A.4; Appendix A). Soil moisture content (end of test) was comparable among all treatments, and was very similar among the reference soil treatments.

8 Discussion

The results of the acute invertebrate testing indicate that the reference site soil amended with barium acetate up to concentrations of 12,000 mg BaAc/kg soil d.w. was not acutely toxic to collembola, but 7- and 14-d LC50s were estimated for earthworms at 7673 and 7168 mg BaAc/kg soil d.w., respectively. The results of the acute screening plant test indicate that the reference site soil amended with barium acetate was acutely toxic to alsike clover with adverse effects observed at concentrations ≥ 1000 mg BaAc/kg soil d.w. and greater.

Generally, the results of the definitive plant testing show that adverse effects were observed at contaminations ≥ 500 mg BaAc/kg soil d.w, for all three species. Alsike clover appeared to be slightly more sensitive than perennial ryegrass or orchard grass but the difference was not great. IC50s for all plant species and endpoints ranged from 529 to 1892 mg BaAc/kg soil d.w.

O. folsomi adult fecundity and reproduction were adversely affected at concentrations between 600 and 1000 mg BaAc/kg soil d.w.. Adult survivorship was adversely affected at contaminations ≥ 6000 mg BaAc/kg soil d.w.

The results of the definitive earthworm testing indicate that the physical and/or chemical properties of the test soil were not conducive to *E. andrei* reproduction. At the treatment level of 1000 mg BaAc/kg soil d.w both adult fecundity and reproduction decreased, relative to the control.

Natalie

Natalie Feisthauer
Project Manager

Gladys L Stephenson

Gladys Stephenson
Project Director

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Appendix A

Results of the Site Soil Assessment: Ecotoxicity Evaluation with Plant and Invertebrate Species

Appendix VIII

Development of Soil Contact Guideline for Extractable Barium

APPENDIX VIII – EXTRACTABLE BARIUM
SOIL CONTACT GUIDELINE

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1. INTRODUCTION

The toxicity data in Stantec (2003a) (Appendix VII) were presented in terms of the nominal concentration of barium acetate added. This Appendix describes how these toxicity data were converted from a nominal barium acetate basis to an extractable barium basis, and how the soil contact guidelines for extractable barium were calculated.

2. METHODOLOGY

2.1 Extractable Barium Regression

Soil sample concentration series from either the beginning or end of five different toxicity tests from the Stantec (2003a) program were submitted to EnviroTest Labs in Saskatoon for extractable barium analysis, according to the protocol described in Section 6.2.2 in the main text. Analytical results are compiled in Table VIII-1 with an explanation of which barium acetate concentration in which toxicity test each sample came from.

Figure VIII-1 shows the measured extractable barium plotted against the nominal concentration of barium acetate for each of the five soil sample series. As can be seen, in Figure VIII-1, the five curves essentially form part of the same data set, and so it appears that the measured concentration of extractable barium is independent of the species that was present in the toxicity test, and whether the sample was collected at the beginning or the end of the test.

Figure VIII-2 shows the regression of the combined data set from all five soil sample series. The regression equation was $y = 0.439x - 25.180$, where x is the nominal concentration of barium acetate, and y is the measured concentration of extractable barium.

2.2 Soil Quality Guideline Calculation

Stantec (2003a) (Appendix VII) provided all their toxicity data on a nominal barium acetate basis. In the current document, the 20th percentile effect data (*i.e.*, IC_{20}, EC_{20}, and LC_{20}) were used to compute the soil contact guidelines, based on guidance in CCME (2006). The nominal 20th percentile data from Stantec (2003a) are summarized in Table VIII-2.

Table VIII-3 shows the results of applying the regression equation from Section 2.1 above to the nominal toxicity data, and summarizes the 20th percentile toxicity data expressed on an extractable barium basis. The different growth endpoints for each plant species were considered redundant, as were the different reproduction endpoints for springtails. Table VIII-3 combines each group of redundant endpoints as their geometric mean (based on guidance in CCME (2006), and calculates the soil contact guidelines for extractable barium. The soil contact guideline for natural areas, agricultural land, and residential/parkland land uses was calculated as the 25th percentile of the geometric means in Table VIII-3. The soil contact guideline for commercial and industrial land uses was calculated as the 50th percentile of the geometric means in Table VIII-3. Again, these procedures followed guidance in CCME (2006).

Table VIII-1. Summary of Extractable Barium Analyses

Stantec Sample Name	Test Species	Sample Collected at Day (d)	Barium Acetate Added (mg/kg)	Extractable Barium Measured (mg/kg)
				Springtail Acute Day 7
O.F. - ACUTE 2 - CONTROL	Springtail	7	0	41
O.F. - ACUTE 2 - 2000	Springtail	7	2,000	673
O.F. - ACUTE 2 - 4000	Springtail	7	4,000	1,419
O.F. - ACUTE 2 - 6000	Springtail	7	6,000	2,456
O.F. - ACUTE 2 - 8000	Springtail	7	8,000	3,461
O.F. - ACUTE 2 - 10000	Springtail	7	10,000	4,270
O.F. - ACUTE 2 - 12000	Springtail	7	12,000	5,337
				Earthworm Acute Day 0
E.A. - ACUTE 2 - CONTROL	Earthworm	0	0	239
E.A. - ACUTE 2 - 2000	Earthworm	0	2,000	691
E.A. - ACUTE 2 - 4000	Earthworm	0	4,000	1,612
E.A. - ACUTE 2 - 6000	Earthworm	0	6,000	2,558
E.A. - ACUTE 2 - 8000	Earthworm	0	8,000	3,459
E.A. - ACUTE 2 - 10000	Earthworm	0	10,000	4,452
E.A. - ACUTE 2 - 12000	Earthworm	0	12,000	5,321
				Invertebrate Reproduction Day 0
E.A./O.F. REPRODUCTION - CONTROL	Invertebrates	0	0	49
E.A./O.F. REPRODUCTION - 10	Invertebrates	0	10	50
E.A./O.F. REPRODUCTION - 30	Invertebrates	0	30	72
E.A./O.F. REPRODUCTION - 60	Invertebrates	0	60	68
E.A./O.F. REPRODUCTION - 100	Invertebrates	0	100	75
E.A./O.F. REPRODUCTION - 300	Invertebrates	0	300	114
E.A./O.F. REPRODUCTION - 600	Invertebrates	0	600	207
E.A./O.F. REPRODUCTION - 1000	Invertebrates	0	1,000	362
E.A./O.F. REPRODUCTION - 3000	Invertebrates	0	3,000	1,203
E.A./O.F. REPRODUCTION - 6000	Invertebrates	0	6,000	2,513
E.A./O.F. REPRODUCTION - 8000	Invertebrates	0	8,000	3,492
E.A./O.F. REPRODUCTION - 10000	Invertebrates	0	10,000	4,450
E.A./O.F. REPRODUCTION - 12000	Invertebrates	0	12,000	5,417
				Alsike Clover Definitive Day 0
ALSIKE CLOVER - DEF - CONTROL	Alsike Clover	0	0	48
ALSIKE CLOVER - DEF - 75	Alsike Clover	0	75	61
ALSIKE CLOVER - DEF - 150	Alsike Clover	0	150	75
ALSIKE CLOVER - DEF - 300	Alsike Clover	0	300	124
ALSIKE CLOVER - DEF - 500	Alsike Clover	0	500	186
ALSIKE CLOVER - DEF - 1000	Alsike Clover	0	1,000	365
ALSIKE CLOVER - DEF - 2000	Alsike Clover	0	2,000	833
				Orchard Grass Definitive Day 14
ORCHARD GRASS - DEF - CONTROL	Orchard Grass	14	0	85
ORCHARD GRASS - DEF - 75	Orchard Grass	14	75	62
ORCHARD GRASS - DEF - 150	Orchard Grass	14	150	80
ORCHARD GRASS - DEF - 300	Orchard Grass	14	300	133
ORCHARD GRASS - DEF - 500	Orchard Grass	14	500	177
ORCHARD GRASS - DEF - 1000	Orchard Grass	14	1,000	348
ORCHARD GRASS - DEF - 2000	Orchard Grass	14	2,000	744

Table VIII-2. Summary of Extractable Barium Analyses Based on Nominal Barium Acetate Concentrations

Effect Measurement	Endpoint	Unit	Alsike Clover	Orchard Grass	Perennial Ryegrass	Springtail Survival	Springtail Reproduction	Earthworm
Test Duration		days	21	14	14	35	35	14
Shoot length	IC20	mg/kg	859	1,105	223	-	-	-
Shoot wet mass	IC20	mg/kg	364	478	181	-	-	-
Shoot dry mass	IC20	mg/kg	294	NA	247	-	-	-
Root length	IC20	mg/kg	653	480	650	-	-	-
Root wet mass	IC20	mg/kg	351	1,000	623	-	-	-
Root dry mass	IC20	mg/kg	475	NA	556	-	-	-
Nodules	IC20	mg/kg	NA	-	-	-	-	-
adult survival	LC20	mg/kg	-	-	-	5,996	-	6000[a]
No. Juveniles	EC20	mg/kg	-	-	-	-	1,158	-
Adult Fecundity	EC20	mg/kg	-	-	-	-	1,174	-

Notes:

NA = not applicable, endpoint could not be calculated

- = not measured

a. LC20 not calculated - NOEC used instead

Table VIII-3. Summary of Extractable Barium Analyses and Soil Contact Guideline Calculation

Data Were Recalculated Using the Following Regression:	**$y = 0.439x - 25.180$**

Effect Measurement	**Endpoint**	**Unit**	**Alsike Clover**	**Orchard Grass**	**Perennial Ryegrass**	**Springtail Survival**	**Springtail Reproduction**	**Earthworm**
Test Duration		**(days)**	**21**	**14**	**14**	**35**	**35**	**14**
Shoot length	IC20	(mg/kg)	402	510	123	-	-	-
Shoot wet mass	IC20	(mg/kg)	185	235	104	-	-	-
Shoot dry mass	IC20	(mg/kg)	154	NA	134	-	-	-
Root length	IC20	(mg/kg)	312	236	311	-	-	-
Root wet mass	IC20	(mg/kg)	179	464	299	-	-	-
Root dry mass	IC20	(mg/kg)	234	NA	269	-	-	-
Nodules	IC20	(mg/kg)	NA	-	-	-	-	-
adult survival	LC20	(mg/kg)	-	-	-	2,657	-	2,659
No. Juveniles	EC20	(mg/kg)	-	-	-	-	534	-
Adult Fecundity	EC20	(mg/kg)	-	-	-	-	540	-
Geometric Mean of Redundant Endpoints		**(mg/kg)**	**230**	**339**	**187**	**2,657**	**537**	**2,659**

Natural Area, Agricultural and Residential/Parkland Guideline (25th Percentile of Geometric Means):	**257**
Commercial and Industrial Guideline (50th Percentile of Geometric Means):	**438**

Notes:

NA = not applicable, endpoint could not be calculated

- = not measured

Figure VIII-1. Extractable Barium Correlations

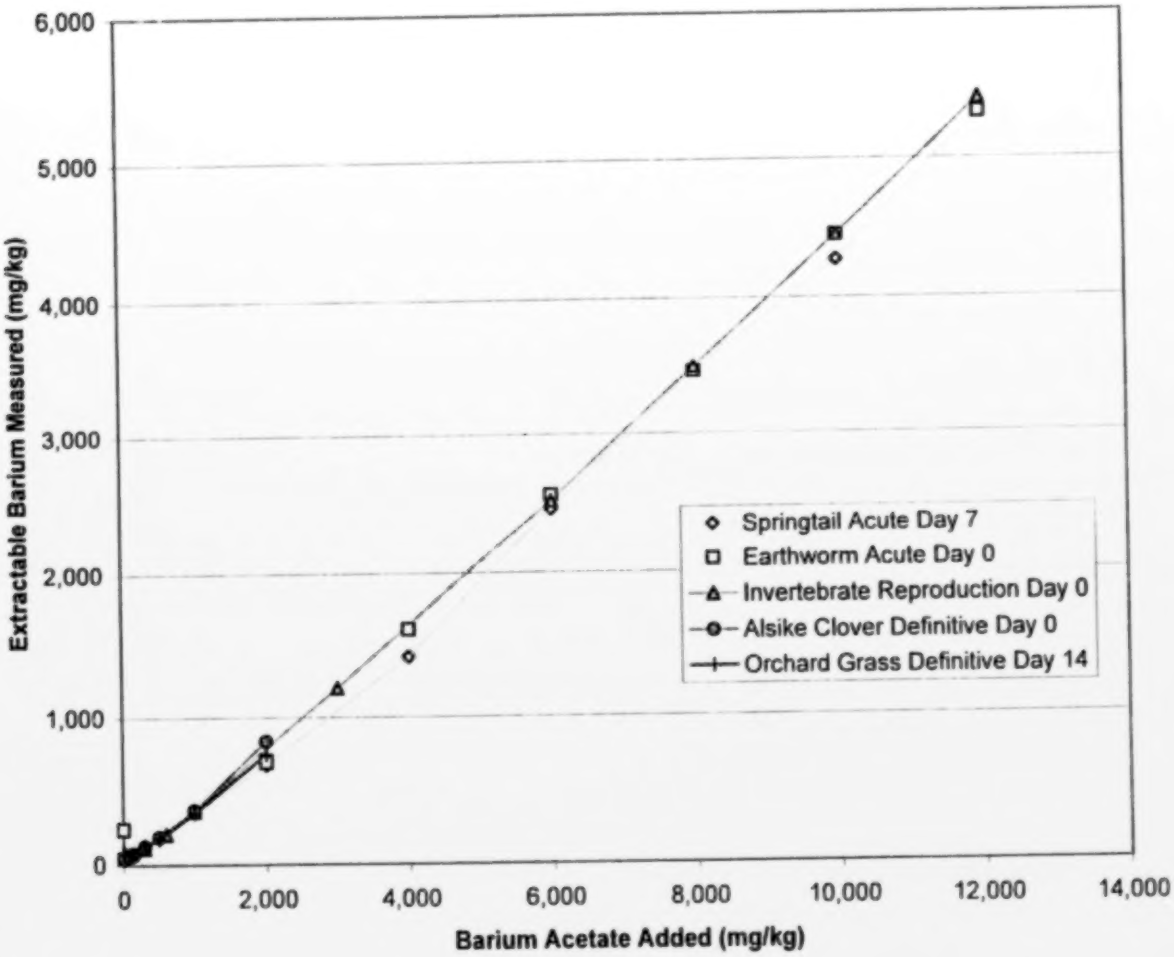

Figure VIII-2. Extractable Barium Regression Using All Available Data